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CLINICAL AND EXPERIMENTAL

ON THE ABSORPTION AND EXCRETION OF METHYLSALICYLATE ADMINISTERED BY INUNCTION*

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PREVIOUS RESEARCH

THE percutaneous absorption of the salicylates, including methylsalicylate, was investigated as early as 1876 by Drasche,[3] who showed that an alcoholic solution of salicylic acid applied to the skin caused an almost immediate appearance of salicylate in the urine. Since then, numerous other investigators[1, 7] have confirmed the rapid percutaneous absorption of salicylic acid. As this drug proved too irritant for inunction, further attention turned to its esters. Several of these were found to be more rapidly absorbed from the skin, such as methyloxymethyl salicylate, glycol salicylate, and particularly the now preferred methylsalicylate (Impens and Sauerland).[6, 8] In some quarters, however, the percutaneous absorption of the methyl ester is still doubted. Goodman and Gilman,[4] for example, propound the following judgment:

> "Methylsalicylate is . . . absorbed when applied cutaneously, but this route is too uncertain when systemic effects are desired."

There may be some justification for such criticism, as the evidence assembled so far has not been convincing. To wit: Sauerland[8] found that only 0.5 per cent of the applied methylsalicylate was absorbed from the skin, though Impens[6] maintained that the amount absorbed was 8 per cent to 9 per cent; Hanzlik and Preshlo[5] found absorption to be considerably delayed; therefore, they judged it to be slow and irregular; certain other investigations appear unconvincing because of the rather artificial methods of application used, such as the immersion of hands in fatty or alcoholic solutions of methylsalicylate, etc. (Brown and Scott).[2]

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INVESTIGATION ON A LARGER SCALE

The study here described was done with a larger number of subjects than those reported by previous investigators. A total of 103 inunctions were made on 86 individuals. The series was large enough to lead us to believe that an over-all average may be established in spite of considerable variation from individual to individual, caused by differences in skin permeability, kidney function, etc. The study was undertaken to determine the salicylate excretion after inunction of methylsalicylate and to compare it with that occurring after the oral administration of one tablet (0.3 Gm.) of acetylsalicylic acid.

VEHICLES USED

Three different methylsalicylate ointments were used in the study. While their salicylate content was the same (20 per cent), different bases were employed as vehicles:

1. Anhydrous lanolin
2. Anhydrous lanolin and menthol, giving the ointment the following composition:

Lanolin	60 per cent
Menthol	20 per cent
Methylsalicylate	20 per cent

3. A special aqueous base containing:

Glycerin monostereate	35.0 per cent
Phenolic resin	4.2 per cent
Acacia	3.5 per cent
Water	28.0 per cent
Alcohol	28.0 per cent
Glycerin	1.3 per cent
Total	100 per cent

This rather solid mixture of base 3 was able to emulsify 20 per cent of its weight of methylsalicylate and 20 per cent of menthol, forming a soft ointment which upon inunction was absorbed by the skin without leaving the skin greasy. The lanolin ointments, by contrast, leave a greasy residue, no matter how long they are rubbed on the skin.

As the presented observations will show, the type of ointment used exerted little influence upon the amount of methylsalicylate absorbed. The amount of ointment used for each inunction, regardless of base, was 10 Gm.

TIME LIMITS OF EXCRETIONS

In order to determine the time over which salicylate is excreted, as well after inunction of methylsalicylate as after oral administration of acetylsalicylic acid, urine samples were collected at short intervals after inunction or ingestion, viz., after ¼ hour, ½ hour, 1 hour, and 2, 4, 8, 12, 18, 24 hours. Each sample was tested qualitatively for the presence of salicylate by the addition of a ferric salt; if the urine sample had to be acidified, a small amount of hydrochloric acid was added.

Eight subjects were used for this qualitative determination with methylsalicylate inunctions. In two of these, excretion was limited to two hours; in five, it occurred over a period of 12 hours; only in one case was excretion protracted more than 12 hours.

With acetylsalicylic acid taken by mouth 33 subjects were used. In 31 of these, salicylate excretion was completed in 12 hours; in the other two, a small residual amount was excreted a few hours later.

For the purpose of determining quantitative excretion it seemed justified therefore to limit the time, over which the urine was to be collected to 12 hours.

QUANTITATIVE DETERMINATION

The subjects employed were medical students whose interest in the study assured the proper carrying out of instructions. Each of the subjects was routinely provided with 10 Gm. of a 20 per cent methylsalicylate ointment and instructed to rub the ointment into the skin of his chest, abdomen, and thigh at 6 P.M. without wasting any part of the ointment. If the skin felt uncomfortably greasy after inunction with the lanolin ointments, the excess could be wiped off one-half hour after application, but not sooner.

TABLE I

AMOUNT OF SALICYLIC ACID (IN MG.) EXCRETED AFTER INUNCTION WITH 2 GM. OF METHYLSALICYLATE CONTAINED IN 10 GM. OF AN OINTMENT CONSISTING OF ANHYDROUS LANOLIN 80 PER CENT AND METHYLSALICYLATE 20 PER CENT

NAME	MG. OF SALICYLIC ACID EXCRETED	DEVIATION FROM AVERAGE	VOLUME OF URINE IN 12 HOURS
de Cruz	47.2	5.6	770 c.c.
Corn	50.5	8.9	700 c.c.
Schaffner	50.3	8.7	295 c.c.
Boettger	40.4	1.2	410 c.c.
Darby	19.8	21.8	200 c.c.
Average:	41.6	9.3*	

*Average positive deviation: 7.7.
Average negative deviation: 11.5.

All urine voided from the time of the inunction until the next morning was to be collected in a vessel provided for the purpose. At least two voidings were to be made. The total urine so collected was measured, and 100 c.c. of it were used for determining the salicylate content. Determination was made according to the method of Thoburn and Hanzlik, as follows:

The urine (100 c.c.) and 20 c.c. of added syrupy phosphoric acid were boiled in a 500 c.c. Erlenmeyer flask over a direct flame. The flask was connected with an attachment for conducting steam through it and also with a condenser. Steam was run through the flask, and the distillation was continued until a few drops of the distillate failed to give a pink color reaction when 1 or 2 drops of iron ammonium sulfate were added. When the contents of the Erlenmeyer flask, during the process of distillation, were almost exhausted, or when they foamed and gave rise to fog, water was added and the distillation continued until the distillate proved free from salicylate.

Iron ammonium sulfate was added to the distillate until the violet color of the reaction could no longer be intensified by further addition of the ferric salt. Salicylic acid was then determined colorimetrically by checking against a stand-

TABLE II

AMOUNT OF SALICYLIC ACID EXCRETED (IN MG.) AFTER INUNCTION WITH 2 GM. OF METHYLSALICYLATE CONTAINED IN 10 GM. OF AN OINTMENT CONSISTING OF ANHYDROUS LANOLIN 60 PER CENT, MENTHOL 20 PER CENT, METHYLSALICYLATE 20 PER CENT

NAME	MG. OF SALICYLIC ACID EXCRETED	DEVIATION FROM AVERAGE	VOLUME OF URINE IN 12 HOURS
Gouldin	43.0	12.1	580 c.c.
Juler	92.6	37.5	486 c.c.
Kearney	70.7	15.6	514 c.c.
Graham	61.0	5.9	334 c.c.
Gan	72.5	17.4	448 c.c.
Snyder	74.8	19.7	943 c.c.
Jurewicz	38.8	16.3	416 c.c.
Minnick	49.0	6.1	428 c.c.
Jablonski	90.5	35.4	1155 c.c.
Rigby	40.6	14.5	850 c.c.
Lyons	54.3	0.8	585 c.c.
Chemycz	43.7	11.4	500 c.c.
Neely	43.8	11.3	800 c.c.
Fedullo	16.8	38.3	250 c.c.
Ingersoll	16.5	38.6	1050 c.c.
Gleason	49.0	6.1	486 c.c.
Griffin	78.4	23.3	700 c.c.
Fritz	104.1	49.0	865 c.c.
Ennis	28.6	26.5	340 c.c.
Yamula	34.0	21.1	440 c.c.
Zaydon	43.0	12.1	800 c.c.
Martucci	66.3	11.2	650 c.c.
Average:	55.1	19.5*	

*Average positive deviation: 23.9.
Average negative deviation: 16.6.

TABLE III

AMOUNT OF SALICYLIC ACID (IN MG.) EXCRETED AFTER INUNCTION WITH 2 GM. OF METHYLSALICYLATE CONTAINED IN 10 GM. OF AN OINTMENT CONSISTING OF SPECIAL ABSORBABLE BASE (SEE TEXT) 60 PER CENT, MENTHOL 20 PER CENT, METHYLSALICYLATE 20 PER CENT

NAME	MG. OF SALICYLIC ACID EXCRETED	DEVIATION FROM AVERAGE	VOLUME OF URINE IN 12 HOURS
Munchak	21.0	26.5	350 c.c.
Chepko	94.1	46.6	467 c.c.
Carney	29.1	18.4	715 c.c.
Calvanese	55.2	7.7	400 c.c.
Ballas	54.4	6.9	514 c.c.
Konopka	37.6	9.9	500 c.c.
Wolf	24.5	23.0	368 c.c.
Mozola	27.4	20.1	740 c.c.
Wasley	57.1	9.6	936 c.c.
Witherspoon	46.3	1.2	755 c.c.
O'Brien	30.0	17.5	370 c.c.
Turner	41.9	5.6	935 c.c.
Montalvo-Carroll	55.7	8.2	1105 c.c.
Sussman	97.3	49.8	450 c.c.
Woelfel	41.0	6.5	484 c.c.
Average:	47.5	17.2*	

*Average positive deviation: 21.5.
Average negative deviation: 14.3.

ard solution containing 0.115 Gm. of sodium salicylate per liter; 1 c.c. of this standard solution corresponds to 0.1 mg. salicylic acid; enough iron ammonium sulfate was added to give the maximal pink color (which remains constant for a week).

The quantity of salicylic acid in the 100 c.c. sample was thus determined and, from it, the total quantity of the acid excreted in the urine after inunction with an

ointment containing 2 Gm. of methylsalicylate. The results obtained are listed in the tables shown.

INFLUENCE OF VEHICLES

The vehicle used to present the salicylate to the integument appeared to be of no influence upon the amount excreted in the urine. For confirmation of this finding a number of inunctions with the different bases were made on the same individuals; again the vehicles appeared to be without significant influence (cf. Table IV). When anhydrous lanolin constituted the vehicle, average excretion was 101.3 mg. of salicylic acid; with the absorbable base, 103.1 mg. were excreted; in both instances menthol (20 per cent) had been added to the ointment. Within the limits of experimental error, these figures (amounts excreted) are obviously identical.

It is interesting to note here that the omission of menthol, when the ointment presented methylsalicylate alone in anhydrous lanolin, resulted in a considerably smaller amount of salicylic acid excreted, an average of 64.6 mg. Apparently the addition of menthol promotes greater absorption, hence greater excretion.

On the whole, all averages in this comparative series of inunctions (made on the same individuals) were higher, because a selected group of subjects was used, men who had shown better cutaneous absorption than the average. Dark-complexioned individuals apparently have a higher absorption ability than the blonds.

The data of urinary excretion after oral administration of acetylsalicylic acid are presented in Table V.

CONCLUSION

Tables I to VI show that the following quantities of salicylic acid were excreted:

From Inunction

	Tables I, II, III	Selected Group Table IV
	mg.	mg.
(a) without menthol in lanolin	41.6	64.6
(b) with menthol in lanolin	55.1	101.3
(c) with menthol in absorbable base	47.5	103.1
Average	48.1	89.7

From Oral Ingestion

acetylsalicylic acid, Table V: 65.8 mg.

It is evident that excretion of salicylate after oral administration of acetylsalicylic acid (average 65.8 mg.) is on the same level as that from cutaneously applied methylsalicylate (averages 48.1 to 89.7 mg.). If the excretion is virtually the same, it is only logical that absorption into the blood stream and the systemic influence exerted also must have been the same. The conclusion ap-

TABLE IV

EXCRETION OF METHYLSALICYLATE IN URINE AFTER INUNCTION WITH 2 GM. OF THIS SALICYLATE CONTAINED IN 10 GM. OF OINTMENTS WITH THREE DIFFERENT VEHICLES IN EIGHT SELECTED INDIVIDUALS

NAME	LANOLIN	DEVIATION FROM AVERAGE	LANOLIN PLUS 20 PER CENT MENTHOL	DEVIATION FROM AVERAGE	ABSORPTION BASE PLUS 20 PER CENT MENTHOL	DEVIATION FROM AVERAGE
de Cruz	114.0 mg.	49.4	150.4 mg.	49.1	90.6 mg.	12.5
Wolf	140.5 mg.	75.9	99.8 mg.	1.5	127.0 mg.	23.9
Weiss	76.2 mg.	11.6	53.8 mg.	47.5	125.0 mg.	21.9
Rubin	35.8 mg.	28.8	43.7 mg.	57.6	44.8 mg.	58.3
Uzman	82.2 mg.	17.6	98.8 mg.	2.5	200.4 mg.	97.3
Davis	4.1 mg.	60.5	138.0 mg.	36.7	164.3 mg.	61.2
Lapinsohn	35.7 mg.	28.9	48.0 mg.	53.3	21.7 mg.	81.4
Baddour	28.0 mg.	36.6	178.0 mg.	76.7	50.8 mg.	52.3
Average:	64.6 mg.	38.7*	101.3 mg.	40.6**	103.1 mg.	51.1***

*Average positive deviation: 38.6.
Average negative deviation: 38.7.

**Average positive deviation: 54.2.
Average negative deviation: 32.5.

***Average positive deviation: 51.1.
Average negative deviation: 51.1.

TABLE V

AMOUNT OF SALICYLIC ACID (IN MG.) EXCRETED AFTER ORAL INGESTION OF 0.3 GM. OF ACETYSALICYLIC ACID

NAME	MILLIGRAMS OF SALICYLIC ACID EXCRETED	DEVIATION FROM AVERAGE	VOLUME OF URINE IN 12 HOURS
Brzoza	65.8	0.0	940 c.c.
Brobyn	53.7	12.1	350 c.c.
Berio-Suarez	97.6	31.8	390 c.c.
Makel	72.0	6.2	390 c.c.
Lee	53.6	12.2	550 c.c.
Musser	25.2	40.6	760 c.c.
Boyd	133.3	67.5	337 c.c.
Schlossmann	32.2	33.6	350 c.c.
Groth	71.7	5.9	600 c.c.
Gambescia	67.5	1.7	2500 c.c.
Rozanski	45.0	20.8	755 c.c.
Goldstein	114.0	48.2	1850 c.c.
Prunetti	79.7	13.9	475 c.c.
Torres-Oliver	56.3	9.5	700 c.c.
Michaile	128.1	62.3	940 c.c.
Shuttleworth	56.0	9.8	1002 c.c.
Raffa	84.5	18.7	957 c.c.
Souder	29.0	36.8	576 c.c.
Boyer	44.0	21.8	1000 c.c.
Blood	120.4	54.6	507 c.c.
Eckroth	49.1	16.7	636 c.c.
Davis	58.8	7.0	1200 c.c.
Drewery	32.9	32.9	888 c.c.
Guito	50.9	14.9	375 c.c.
Berry	93.7	27.9	422 c.c.
Esgro	48.0	17.8	2000 c.c.
Castagna	60.6	5.2	600 c.c.
Witman	80.2	14.4	750 c.c.
Pigozzi	68.0	2.2	500 c.c.
Kane	37.5	28.3	750 c.c.
Leedom	90.5	24.7	875 c.c.
Flinkman	46.2	19.6	700 c.c.
Galamaga	26.3	39.5	750 c.c.
Average:	65.8	23.0*	

*Average positive deviation: 27.1.
Average negative deviation: 20.0.

TABLE VI

IN A FEW CASES, METHYLSALICYLATE (0.3 GM. IN A CAPSULE) WAS ADMINISTERED ORALLY AND THE EXCRETION STUDIED. AS THE FOLLOWING FIGURES SHOW, THE SALICYLATE EXCRETION WAS APPROXIMATELY AT THE SAME LEVEL AS AFTER ORALLY INGESTED ACETYLSALICYLIC ACID.

NAME	MG. OF SALICYLIC ACID EXCRETED	DEVIATION FROM AVERAGE	VOLUME OF URINE IN 12 HRS.
Mason	128.6	33.0	1180 c.c.
Gambone	42.0	53.6	500 c.c.
Davis	58.8	36.8	1200 c.c.
Thomas	152.4	56.8	1270 c.c.
Average:	95.6	45.1*	

*Average positive deviation: 44.9.
Average negative deviation: 45.2.

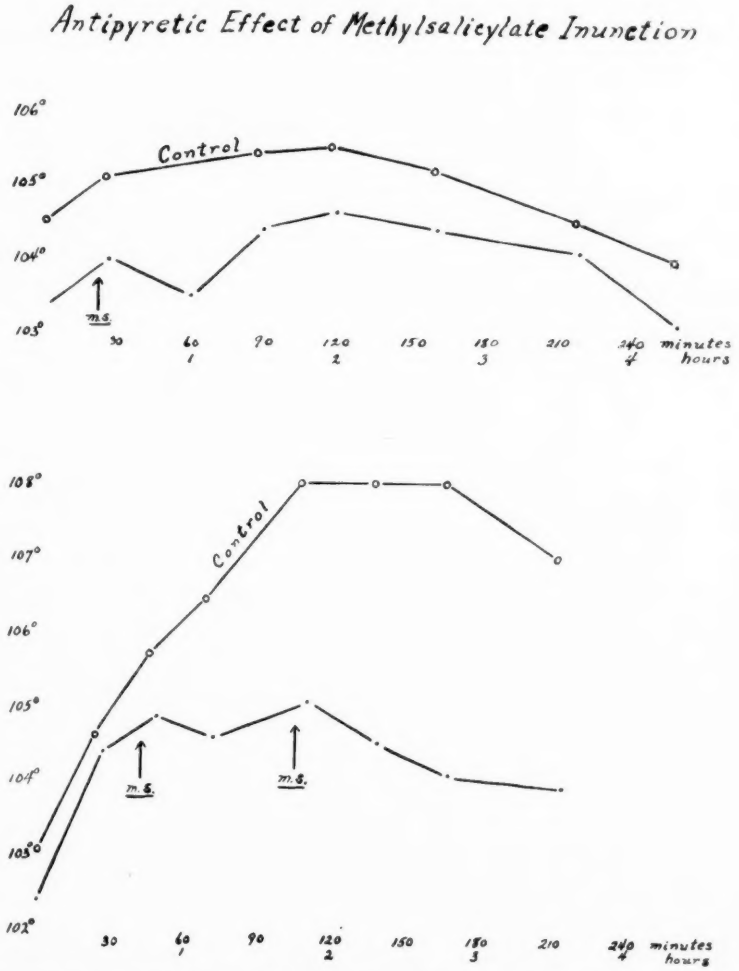

Fig. 1.—In each of these two sets of experiments two rabbits were used; both were injected with 20 mg./kg. of cocaine hydrochloride, whereupon the rectal temperature gradually rose, later slowly returning to normal as indicated. After the application of a 20 per cent methylsalicylate ointment to the ear of one of the animals, however, *m.s.* on graph, a prompt drop of temperature was seen. This drop may be the result of central action of methylsalicylate on the (disturbed) heat center, since we have shown that sizable amounts of this salicylate are absorbed through the skin, or it may be due to a vasodilation resulting from the local irritating effect exerted by the drug. Or both types of action may contribute to the lowering of temperature.

pears justified, therefore, that an adequate inunction of methylsalicylate ointments, of the types described, must be considered internal medication.

ANTIPYRETIC AND VASODILATING EFFECT OF METHYLSALICYLATE

In order to test whether methylsalicylate upon cutaneous application exerts a systemic influence, its antipyretic effect was investigated. Rabbits were injected with cocaine in a quantity of 20 mg. per kilogram of body weight. Within two hours, the temperature of the injected animals gradually rose from approximately 103° F. to approximately 106° F., then slowly returned to normal. When methylsalicylate ointment was rubbed into the ears of the animals, the temperature dropped promptly (see Figs. 1 and 2). This effect may, of course, have been merely the result of the vasodilation produced locally by methylsalicylate, which is a counterirritant.

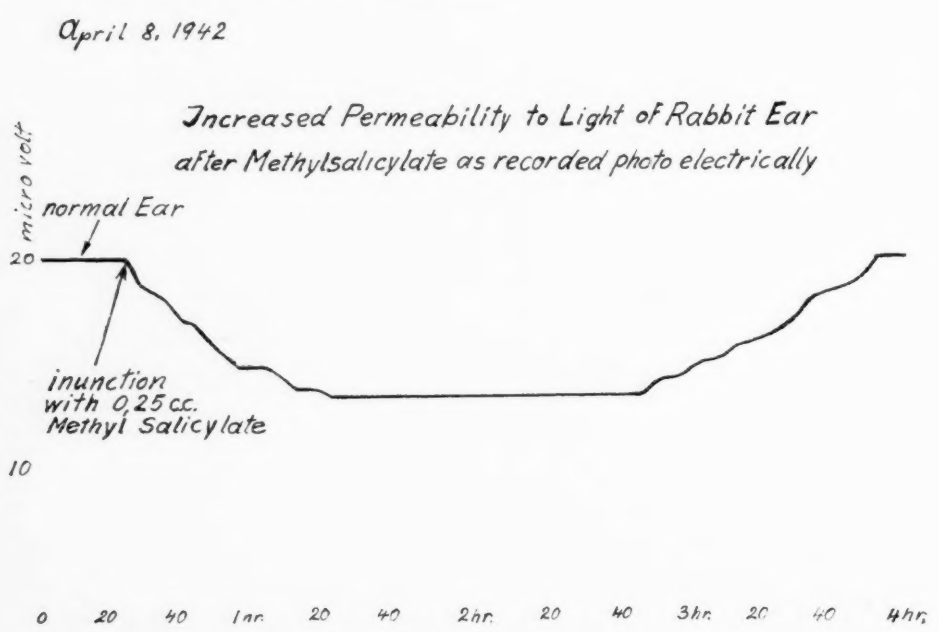

Fig. 2.—The hyperemia resulting from local application of methylsalicylate on the rabbit's ear was studied photoelectrically. The ear was held in a frame close to a photoelectric cell (RCA high vacuum phototube 917). The light from a small 3 volt bulb, fed by a 2.5 volt battery was allowed to fall on the rabbit's ear; some of it passed through the translucent tissue and elicited a feeble photoelectric current of the phototube, the voltage of which was recorded continuously on a Leeds and Northrop "Micromax Recorder." The accompanying graph is a twofold magnification of the record thus obtained.

At the point indicated on the graph, the rabbit's ear was rubbed gently with two drops of methylsalicylate, whereupon the photoelectric current decreased due to the greater opaqueness from hyperemia through irritation by the methylsalicylate. Mere mechanical rubbing without a drug had no such effect. (Performed by Mr. George Callé.)

This well-known property of the drug was again demonstrated by the increased ability of a rabbit's ear, after inunction, to transmit light, as measured photoelectrically (see Fig. 2).

SUMMARY

The absorption and excretion of cutaneously applied methylsalicylate was tested in 83 individuals on whom a total of 103 inunctions were made.

Findings were compared with those derived from the oral administration of acetylsalicylic acid.

Similarity of the derived figures (averages) justifies the conclusion that methylsalicylate upon cutaneous application exerts not only its long-established counterirritant influence (vasodilatation, decongestion, induction of active hyperemia), but also a systemic action which perforce must be analgesic, antipyretic, and antirheumatic, since these properties are common to the salicylates upon absorption.

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SYSTEMIC ALLERGIC REACTION INDUCED BY YELLOW FEVER VACCINE

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THIS is the record of a case of severe constitutional reaction to the single immunization injection of yellow fever vaccine. It is not the purpose of the writer to detract in the least from the established value of this most efficacious prophylactic agent. In an exhaustive review of the literature, not a single similar case was discovered. When it is considered that upward of several million immunizing injections of yellow fever vaccine have been given to date, the rarity of the case reported below is obvious. It is undoubtedly true that many such reactions of much less severity have been seen and have gone unrecognized or have been recognized and considered of no importance because of their mildness.

Three cases resembling the writer's in minor aspects have been reported by Sulzberger and Asher.[1] These were cases of urticaria and erythema multiforme-like eruptions appearing thirty-six or more hours after yellow fever vaccine injections. Accompanyng the skin manifestations were arthralgias, malaise, fever, pruritus, nausea, and vomiting. As the authors state, the entire symptom complex simulated serum sickness rather than the immediate constitutional reaction of atopy.

It is the intention of the writer to point out by example the necessity of knowing, generally, the constitution of the material injected and of the patient himself.

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REPORT OF CASE

A 27-year-old white male was admitted to the hospital on October 15, 1942, for observation. About one month before admission, he received a single injection of cholera vaccine and one of yellow fever vaccine. Less than five minutes after these injections, while making his way out of the clinic, he became acutely ill. His vision blurred; his breathing became labored and extremely difficult; nausea, vomiting, diarrhea followed and generalized edema of the skin upon which appeared large, raised, pruritic, erythematous wheals. The dyspnea increased in severity, and in a short time he lost consciousness.

After repeated subcutaneous injections of epinephrine, the patient regained consciousness. He was then hospitalized; the severe dyspnea, angioneurotic edema, urticaria, and the gastrointestinal symptoms continued. Epinephrine subcutaneously and ephedrine sulfate orally were maintained in decreasing frequency for the next six days, at which time all symptoms disappeared. He was then discharged from the hospital.

Two days later, while engaged in strenuous physical activity, he experienced a severe paroxysm of dyspnea which lasted two hours. In milder form, it continued for two weeks accompanied by nocturnal wheezy respiration. He was again hospitalized for four days, during which time bronchial symptoms persisted. In addition, a mild generalized urticaria, bullous in character, was noticed. Symptoms subsided and he was transferred to this hospital.

The past history of this patient is of special significance. At the age of four, he contracted the "flux," during which illness he was fed large quantities of egg and egg white. From that time on, he was unable to eat eggs. His inability was initiated not only by a marked aversion for them, but also by the knowledge that ingestion of the smallest fraction of an egg or egg-containing food would precipitate severe asthmatic paroxysms. These paroxysms, he noticed, would also appear on close contact with feathers, e.g., sleeping on a feather pillow or working in the chicken coops on the farm where he lived. In addition to asthma, eggs in any form and in the minutest quantity produced nausea, vomiting, diarrhea, swelling of the face, arms, legs, and generalized itchy wheals. As he grew older, other foods produced the same variety of symptoms in somewhat lesser degree. Those he was definitely able to indict were chicken, pork, chocolate, and peanut. He also noticed that the asthma which had become an almost constant but mild characteristic of his life was most marked during the fall and winter seasons. (His home was in Tennessee.) About six years ago, the attacks decreased in frequency and severity, appearing only at long intervals.

Five years ago, he received a single injection of a vaccine as protection against "flu." A few minutes later, an episode similar to the one described above followed. It was treated with epinephrine and subsided in several hours. The nature of the vaccine could not be determined.

The family history was essentially negative except for his father who had had asthma since childhood.

Physical examination on October 15, 1942, revealed a well-developed, white male, sixty-nine inches tall, weighing one hundred and sixty-five pounds with no significant abnormal physical signs. The blood picture and chemistry, urinalysis, serology showed no abnormalities. The EKG was normal. X-ray of the sinuses showed clouding of the left frontal; others were normal. X-ray of the lungs and chest wall showed no abnormalities.

The allergy investigation, which was as exhaustive as facilities permitted, revealed interesting findings. No precise laboratory investigations were instituted so that the specific modified responsible allergen could not be determined; however, intradermal tests, direct and indirect (passive transfer), and clinical tests were made. All of these pointed incontrovertibly to a single solution of the problem: what was the agent that produced the severe constitutional reaction in this patient? The writer deems it best for clarity to develop these investigations apart from the body of the case report.

The history of this patient, which immediately labelled him an atopic individual with a multivalent sensitivity including a persistent, marked egg

and chicken sensitivity, presented an almost immediate theoretical answer to the severe, near-fatal constitutional reaction following injection of the two vaccines. Yellow fever vaccine was prepared by inoculating eggs incubated seven to eleven days with the 17-D strain of attenuated yellow fever virus developed by Theiler and Smith. This was followed by three more days of incubation, at which time the still-living embryos were aseptically removed from their shells, whatever albumin attached to the embryo not being disturbed. They were ground to a fine pulp in blenders cooled with dry ice, diluted with normal human serum or distilled water, centrifuged for thirty to sixty minutes in an angle centrifuge at about 3,500 revolutions per minute to remove tissue debris. The supernatant extract constituted the vaccine. This was evaporated at a low temperature, standardized, and kept at 4° C. When needed it was dissolved in ten volumes of sterile distilled water and given immediately in 0.5 c.c. doses subcutaneously.[2, 3]

The patient was tested intradermally to more than one hundred allergens. Many inhalant and ingestant skin sensitivities were found. Especially marked reactions were obtained to egg white, chicken meat, and yellow fever vaccine undiluted (four plus). Yellow fever vaccine diluted 1:10 produced a two plus reaction. Cholera vaccine was negative at the end of ten minutes, twenty-four hours, and forty-eight hours. Thus far, proof had been obtained of specific skin sensitivity which confirmed his history of atopy. Because cholera vaccine gave a negative skin reaction both immediate and delayed and because yellow fever vaccine undiluted and diluted 1:10 gave positive skin reactions of the immediate variety, it was established that: (1) cholera vaccine was in no way involved in the constitutional reaction that eventually brought the patient to this hospital, and (2) yellow fever vaccine was undoubtedly the instigating agent.

Clinical tests with the patient's permission both with and without his knowledge (the latter to rule out psychogenesis of resulting symptoms) were performed under close supervision. Both egg and chicken meat were used. Symptoms were reproduced more marked to egg than to chicken meat, mild in degree, controlled with epinephrine.

Clinical sensitivity having been established, it remained only to determine which fraction of the yellow fever vaccine was responsible for initiation of symptoms, the virus fraction or the culture medium fraction. In order to do this, several c.c. of the patient's serum were prepared for passive transfer. The volunteer subject was tested intradermally with the full complement of allergens (more than one hundred), and was found negative to all. Twenty sites on the subject's arm were sensitized, each with one-tenth c.c. of the patient's serum. Forty-eight hours later, these sites were tested with twenty different allergens. At twenty-four-hour intervals, the sites were tested with different series of twenty allergens, until all of those which had produced positive reactions on the patient's skin were tested in this manner. Most of these gave positive reactions on the sensitized sites to lesser degree. Egg transferred four plus; chicken meat two plus; yellow fever vaccine undiluted two plus; yellow fever vaccine 1:10 one plus. All of these reactions, of course, were of the immediate variety. This demonstrated the evident fact that there existed in the serum of the patient (now in the skin of the subject's arm)

reagins for egg, chicken meat, and yellow fever vaccine. Since a virus is not known to be reaginogenic nor is it known to produce skin reactions of the immediate variety, the obvious deduction follows that it was the culture medium fraction of yellow fever vaccine that called forth reaginogenesis.

It was now left to relate this fraction constitutionally and reaginogenically to either egg white, chicken meat, or both. Three widely separated transfer sites were chosen. These were exhausted of egg reagin by successive reinjection with egg extract until no reaction was obtained. These sites were then tested with chicken meat extract. A positive reaction was obtained. When this had disappeared and an hour had been allowed to elapse, these same sites were tested with yellow fever vaccine undiluted. A slight reaction, less than one plus, resulted.

Three different sites were then chosen, again widely separated. These were exhausted of chicken meat reagin in the same manner as above. The sites were then tested with egg extract; a four plus reaction resulted. When the reaction had subsided, an hour was allowed to elapse and the same sites were tested with yellow fever vaccine undiluted. Positive reactions resulted only slightly less marked than the reactions in the sites where neither egg nor chicken meat reagin had been exhausted.

This is conclusive evidence that the reaginic fraction of yellow fever vaccine is related both to egg white and chicken meat structurally and reaginogenically; that it more closely resembles egg white than it does chicken meat. It is known that, from the point of view of both clinical and skin sensitivity, egg white and chicken meat are totally unrelated. In the embryonic development of the chick from the egg, a mutation and differentiation of egg protein take place. It is conceivable that the reaginic fraction of yellow fever vaccine (chick embryo culture) represents a stage in this mutation and therefore by test reveals a kinship to both egg white and chicken meat, albeit the relationship is closer with egg white.

Berger and Hargett,[3] working on the problem of anaphylaxis in guinea pigs following sensitization with chick embryo yellow fever vaccine and normal chick embryo, state, "Chick embryo protein derived from embryos of different ages possesses the power to produce anaphylactic sensitization in young guinea pigs in direct ratio to the age of the embryos employed. . . . ." If one were to transpose this work in the laboratory to the clinical and investigative observations made in this case, the variance would be seen immediately. According to the above authors, the reaginic fraction of yellow fever vaccine should be more closely related to chicken meat than to egg white. This cannot be borne out by the writer's findings.

COMMENT

The presentation of this case of an egg-chicken meat sensitive atopic individual who experienced a severe constitutional reaction immediately following injection of yellow fever vaccine is made to point out the necessity of knowing something of the constitution of the immunizing injection and the subject to whom it is to be given. This emphasizes, by example, the instructions given medical officers of the army relative to questioning the subject about allergic history before administering a vaccine. It is not meant to detract in the least from the justified use of this prophylactic agent.

Evidence is offered in proof of the reaginicity of yellow fever vaccine culture medium and of its close relationship to egg white and chicken meat, more marked to egg white.

It is suggested that marked sensitivity by history or skin test to egg, chicken meat, or both, and/or by skin test to yellow fever vaccine is an indication to give the vaccine carefully, in divided doses with epinephrine at hand in the event a constitutional reaction occurs.

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THE PHARMACOLOGY OF TWO WATER-SOLUBLE VITAMIN K-LIKE SUBSTANCES*

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THE object of this study was to test the vitamin K activity, general pharmacology, and toxicity of 2-methyl-1,4-naphthohydroquinone diphosphoric acid ester tetrasodium salt $6H_2O$ (N-123)† and 2-methyl-1,4-naphthohydroquinone sodium bisulfite complex (MNSS).‡ A preliminary report of this work was presented at the Chicago meeting of The Federation of American Societies for Experimental Biology and Medicine, 1941.[1] Experiments reported here were carried out in the laboratories of Northwestern University and Hoffmann-La Roche and the separate results are designated NU and HLR, respectively. This distinction is made on account of the slightly varying methods and results of the two laboratories.

I. VITAMIN K ACTIVITY

Tests were made on hypoprothrombinemic rats by (a) "Petrolagar" diet;[2] (b) Bile duct obstruction after a preliminary depletion with a diet of low vitamin K content.[3, 4] After depletion, the prothrombin deficient rats were treated with subcutaneous injections of N-123 and MNSS.

METHODS

Production of Deficiency (NU).—Petrolagar diet.—Ninety stock rats weighing from 110 to 230 Gm. were placed on a diet consisting of 70 per cent

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†Synkayvite, Hoffmann-La Roche, Inc.

‡Hykinone, Abbott Laboratories, Inc. This substance is an addition product rather than the -3-sodium sulfonate originally reported.[13]

powdered Purina Dog Checkers and 30 per cent Petrolagar. Significant hypoprothrombinemia* developed in 25 per cent of surviving rats in 8 to 96 days as shown by the Quick prothrombin time method.[6] Thirty-two of these rats died in the course of an epidemic of infectious middle ear disease, which swept the colony 30 days after beginning the diet. Thirty-five additional rats were then placed on the diet for a shorter period; of these approximately 50 per cent showed a prolongation of prothrombin time to double or more the original value within 38 days. *Biliary obstruction:* Forty rats were placed on a diet low in vitamin K[5] for periods ranging from 32 to 44 days. In 32 of these the common bile duct was doubly ligated and cut under ether anesthesia. Of 23 rats surviving repeated cardiac puncture and biliary obstruction, 9 or 39 per cent showed a marked hypoprothrombinemia 3 to 10 days following ligation of the common bile duct. During the dietary period there were no significant changes in prothrombin times.

Production† of Deficiency (HLR).—Oil and Oil-Agar Diets.—Petrolagar and later Afkol (a similar preparation) were used, mixing 30 per cent in powdered Purina Dog Chow. Also, 20 per cent mineral oil in the Purina food was used since it kept better and did not mold. Since only a few rats developed significant hypoprothrombinemia, the diet method was abandoned. *Biliary obstruction:* The bile duct was doubly ligated and sectioned. Rats previously on the oil or oil-agar and Purina diet for two months or longer developed prothrombin deficiency in 8 or 10 days. Rats placed on the diet only after operation required 2 to 3 weeks to develop the deficiency. All animals were continued on the diet postoperatively. Seventy-two rats were successfully operated. Of these, 28 died within 2 or 3 weeks though none within the first day or so. Thirty-one deficient rats died following cardiac puncture.

Prothrombin Determination.—Under ether anesthesia 0.45 c.c. of blood was obtained by cardiac puncture (27 gage needle) and drawn into 0.05 c.c. of sodium oxalate solution (1.34 per cent); to this was added 0.5 c.c. physiologic saline, and the specimen was centrifuged. The coagulation time of the plasma was determined by a modified Quick method.[6] To 0.1 c.c. of oxalated plasma at 38° C., 0.1 c.c. of thromboplastin‡ (Quick[7]) was added and quickly thereafter 0.1 c.c. of calcium chloride solution (0.27 per cent). Clotting time was noted with a stopwatch and at least two determinations were run on each sample.

Method of Treatment (NU).—When the prothrombin time had increased significantly (at least twice original value) the animals were injected subcutaneously for two days or more with 2 and 4 gamma doses of both compounds. Prothrombin time (PT) was determined daily. Control animals were injected with physiologic saline, and both substances were dissolved in saline and injected in volumes of 0.5 or 1.0 c.c.

Method of Treatment (HLR).—Depleted animals were injected with single doses ranging from ¼ gamma to 2 gamma per rat. Prothrombin times were determined 18 to 20 hours later. Since the HLR results are reported in terms of per cent prothrombin, a curve relating prothrombin time to per cent pro-

*Prothrombin time increased to twice or more the original level.

†American Pharmaceutical Co., Inc.

‡The dried rabbit brain was placed in glass ampoules sealed under vacuum and appeared to keep indefinitely.

thrombin was determined by plotting data obtained from tests on serial dilutions of plasmas from five normal rats, following the method of Quick.[6]

RESULTS

Results (NU).—Controls.—The mean control reading of prothrombin time in 144 normal rats (both on dietary rats and bile duct obstructed rats) was 22.9 seconds ±0.16 second (S. E. of mean) of ±1.9 seconds (S. D.).

Table I shows the daily PT determinations of 13 rats receiving 0.5 or 1 c.c.

TABLE I

DAILY INJECTION OF SALINE

RAT* NO.	PT** BEFORE DIET	DAYS ON DIET	DAYS POST-OP.	PT BEFORE INJ.	VOLUME INJ. CC.	PT 24 HRS. AFTER 1ST INJ.	PT 24 HRS. AFTER 2ND INJ.	PT 24 HRS. AFTER 3RD INJ.	PT 24 HRS. AFTER 4TH INJ.
P_1	20.7	61		48.6	0.5	47.9	43.9	37.3	
P_2	18.4	28		41.4	0.5	42.0	54.0	43.1	
P_3	22.4	38		41.6	0.5	28.6	28.8	28.6	
P_4	17.8	42		53.2	0.5	47.7	37.4		
P_5	20.2	42		40.7	0.5	51.3	44.8		
P_2	18.4	45		42.0	1.0	52.6	47.3	55.4	63.9
P_6	23.6	34		59.6	1.0	67.8 (Died)			
P_7	21.8	45		44.6	0.5	39.2	35.7	40.2	
P_8	20.6	42		39.8	0.5	39.3	38.5	52.0	
P_9	19.0	92		42.9	1.0	33.8	23.4		
P_{10}	24.3	92		49.2	0.5	50.2	37.2	44.6	
B_1	17.3	44	9	48.0	0.5	48.0	90.0	74.2	91.2
B_2	21.6	36	24	38.8	0.5	38.8	41.7 (Died)		
B_3	19.3	43	11	51.6	0.5	51.6	36.8	47.1	42.6
Mean 20.4				45.72		45.62	43.03	46.94	
Standard error mean				±1.67		±2.71	±4.82	±5.06	

*P = Petrolagar rat; B = Bile duct obstructed rat; the diet in this case was a low vitamin K diet, not Petrolagar.

**P = Prothrombin Time in seconds.

injections of physiologic saline. It is seen that the PT shows rather marked variations from day to day, and in three cases, rats B_3, P_3 and P_9, the injection of saline was accompanied by a considerable reduction of the prothrombin time. Such remissions are thought to be fortuitous and perhaps explainable on daily variations in intestinal absorption. Also, small variations in the vitamin K absorbed are likely to produce magnified changes in prothrombin time when the blood prothrombin level is low, because of the steep slope of the prothrombin time/per cent prothrombin curve in this region. Statistical analysis, however, reveals no significant differences between the mean values for successive days in this control series.

Treated.—In Table II are listed the responses to daily subcutaneous injections of 4 gamma of N-123. The critical ratio (''Student's'' ''t'') between PT before injection and 24 hours after the first injection is 5.03 and between PT before injection and 24 hours after the second injection is 6.32, thus being in both instances highly significant ($P < 0.0002$).

In Table II are shown the responses to subcutaneous injections of 4 gamma MNSS. The critical ratio of the mean PT before injection and after injection is very high (over 6). The critical ratio between the mean value after the first injection and the mean after the second injection is 1.50 (or $P = 0.15$), so most

of the effect of vitamin K was obtained in the first 24 hours. Also in Table II is shown the response of the PT to 2 gamma doses of N-123 and MNSS, respectively.

Storage of Vitamin K in the Body (NU).—In the course of our study, 15 PT determinations were made on 12 rats at various times *after* the deficiency had already been corrected with a series of injections. The results are shown in Table III.

The results show that when the prothrombin time is brought nearly to normal, at 5 days after the last injection it is practically back to the deficient level. These animals received doses of the vitamin K preparations totaling 8 gamma in most cases.

TABLE II

SUMMARY OF RESULTS (NU) ON COMPARATIVE THERAPEUTIC EFFECT OF N-123 AND MNSS

NO. OF RATS	PT BEFORE DIET	PT BEFORE INJ.	PT 24 HR. AFTER 1ST INJ.	PT. 24 HR. AFTER 2ND INJ.	PT. 24 HR. AFTER 3RD INJ.	
11P,* 3B* 14 rats	20.4	45.72 ±1.67	45.62 ±2.71	43.03 ±4.82	46.94 ±5.06	NU, Saline Controls
7P, 6B 13 rats	23.7	57.50 ±3.92	32.31 ±3.21	31.32 ±2.73		NU, 4 gamma N-123
8P, 1B 9 rats	22.5	56.73 ±3.93	31.38 ±1.60	28.45 ±1.12		NU, 4 gamma MNSS
5P, 2B 7 rats	19.4	122.58	37.98 ±3.13	33.18 ±2.65	36.2	NU, 2 gamma N-123
3P, 2B 5 rats	21.4	73.40	43.42 ±3.44	36.74 ±3.08	33.64	NU, 2 gamma MNSS

*P = Petrolagar rats; B = Bile duct obstructed rat.

TABLE III

RETURN OF HYPOPROTHROMBINEMIA FOLLOWING CURE

RAT NO.	PT BEFORE DIET	PT BEFORE INJ.	PT 1 DAY AFTER LAST INJ.	DAYS AFTER LAST INJ.	PT
P_{17}	20.9	45.1	23.6	2	26.9
P_{14}	22.2	44.0	26.9	3	23.0
P_{15}	26.3	58.1	36.5	3	24.7
B_4	23.9	53.4	23.8	3	31.1
B_6	22.6	55.5	28.4	5	48.4
B_4	23.9	41.9	20.3	5	34.9
B_1	17.3	45.8	26.4	5	48.0
P_{28}	21.2	44.8	27.3	6	39.1
P_{29}	17.2	41.3	31.5	6	59.1
B_4	23.9	53.4	23.8	7	54.0
B_4	23.9	54.0	29.2	10	46.2
B_4	23.9	41.9	20.3	10	53.4
B_6	22.6	48.4	35.7	11	42.5
P_{24}	17.2	48.2	27.1	20	34.2
Mean	21.9	48.3	27.2		

Results (HLR).—The mean of 39 PT determinations on normal rats was 21.8 seconds ±0.4 second (S.E. of mean). The standard deviation of a single observation was ±2.5 seconds. These values check well with the NU values.

Injections of N-123 and MNSS were made subcutaneously in deficient rats weighing 150 to 200 grams and PT tests made 18 to 20 hours later. The doses ranged from ¼ to 2 gamma per rat. All deficient rats were markedly jaundiced

and their plasmas were yellow. The results in terms of per cent prothrombin are shown in Table IV. With doses of 2 gamma there was a tendency for the PT to return to a level below 21.8 seconds; that is, the *calculated* per cent prothrombin became higher than normal. This phenomenon does not occur in vitamin K-treated normal animals, however.

When the data are graphed (Fig. 1), it may be noted that with higher doses the two curves approach each other, but that in the lower range there is greater divergence. Doses have been estimated from the curves which give specific effects and these are tabulated in Table V. In addition the relative potencies by weight and molecular weight are given in the latter table. The data in this table demonstrate that on a molecular basis N-123 is 41 per cent more potent than MNSS although on a weight basis it is 26 per cent less potent. These data indicate by indirect comparison that MNSS and 2-methyl-1,4-naphthoquinone (MeNQ) have about equal molecular potencies, since Lee et al.,[8] and Almquist and Klose,[10] respectively, reported N-123 to be 48 per cent and 51 per cent more potent on a molecular basis than MeNQ.

TABLE IV

EFFECT OF VARYING DOSES OF N-123 AND MNSS ON VITAMIN K-DEFICIENT RATS

DOSE GAMMA/RAT	N-123			MNSS		
	N	PER CENT PROTHROMBIN		N	PER CENT PROTHROMBIN	
		BEFORE	AFTER		BEFORE	AFTER
1/4	5	33	39	6	38	52
1/2	3	35	67	2	41	80
1	5	31	68	5	39	87
2	2	37	>100	3	37	>100

TABLE V

RELATIVE POTENCIES OF N-123 AND MNSS IN CORRECTING VITAMIN K-DEFICIENCY IN RATS

INCREASE IN PROTHROMBIN PER CENT LEVEL	CORRESPONDING DOSES (ESTIMATED FROM FIG. 2) IN GAMMA		POTENCY OF N-123 RELATIVE TO MNSS (= 1)	
	N-123	MNSS	BY WT.	BY MOL. WT.
15	0.33	0.25	0.77	1.46
20	0.39	0.28	0.69	1.33
30	0.55	0.39	0.71	1.36
40	0.85	0.59	0.70	1.33
50	1.34	1.10	0.82	1.58
	Average		0.74	1.41

On the Mechanism of Vitamin K Action.—While it is now accepted that vitamin K acts by stimulating the production of prothrombin in the liver, the possibility suggested itself, especially earlier, that there might be a direct action in the blood. Particularly because of the greater molecular potency of N-123 over all known vitamin K-like substances, it was deemed advisable to perform some tests in vitro.

Deficient rats whose prothrombin times varied from 30 to 43 seconds were used. Oxalated whole blood and oxalated plasma were obtained and incubated at 37° C. for ½ to 6 hours with amounts of N-123 varying from 0.1 gamma to 10 gamma per 0.1 c.c. Then the regular prothrombin determinations were made. There were no significant changes in the prothrombin time with either

plasma or whole blood. This observation was confirmed, using blood from 2 patients with hypoprothrombinemia and jaundice.

DISCUSSION

These results indicate that both water-soluble substances are capable of correcting prothrombin deficiencies in rats at dose levels of 2 to 4 gamma per day. At these dose levels the results in the Northwestern University laboratory indicated no significant difference between the two compounds (Table II). Since the ratio of their molecular weights is 530:276, this would indicate that N-123 is about twice as potent as MNSS on a molecular basis; however, the data from the Hoffmann-La Roche laboratory indicated only 50 per cent greater potency by molecular weight.

Since it has been established[8, 10] that the molecular ratio of potency, N-123:MeNQ is about 1.50, it follows from Table V that the molecular ratio of potency, MNSS:NeNQ is 1.50:1.41 or 1.06. While this indirect comparison is only an approximation, it suggests that MNSS and MeNQ are probably of about equal potency on a molecular weight basis.

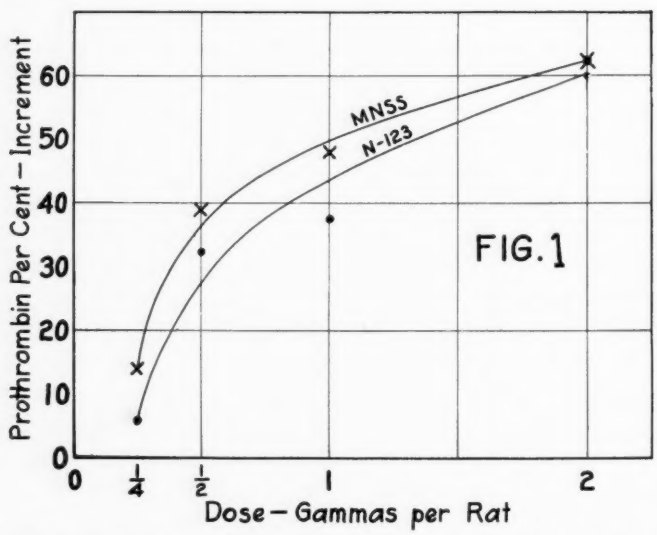

Fig. 1.—Dose-effect curves for N-123 (●) and MNSS (×). Ordinate represents the rise in per cent prothrombin, not the actual per cent prothrombin.

The above ratio, N-123:MeNQ = 1.50, is based on chick assays (both oral feeding and subcutaneous injection). The activity of MeNQ on rats was studied by Flynn and Warner[3] who gave 2 gamma MeNQ daily for two days to bile duct obstructed rats and raised the prothrombin level from 10 to 82 per cent. While the indirect comparisons of parallel results in different laboratories is perhaps equivocal, the Flynn and Warner result is roughly comparable to that of the 2 gamma dose of the two water-soluble substances in Table IV or to the 4 gamma dose in Table II. Taking the latter as the least favorable of the two comparisons, the potency ratio for rats, N-123:MeNQ, is still 1:2 by weight or about 1.54 by molecular weight. This remains to be verified by direct comparison.

II. TOXICOLOGY AND PHARMACOLOGY

Acute Toxicity (NU).—Acute lethal doses were determined by single dorsal subcutaneous injections in albino rats. The animals weighed from 155 to 310 Gm. and there were a few more female than male rats. The results are shown in Table VI. By plotting these data on logarithmic probability paper the LD50's were found to be 610 mg./kg. for N-123 and 175 for MNSS. The ratio, N-123:MNSS, of doses by weight is 3.48, but on a molecular basis (N-123 = 530 and MNSS = 276) the ratio of lethal doses is 1.82.

In a previous communication by one of us[9] the LD50 for N-123 was reported to be 450 mg./kg. in mice by subcutaneous administration and 675 mg./kg. in chicks by the same route.

TABLE VI

TOXICITY BY SUBCUTANEOUS INJECTION IN RATS (NU)

SUBSTANCE	DOSE MG./KG.	N	PER CENT MORTALITY
N-123	450	10	20
N-123	550	10	30
N-123	650	10	60
MNSS	100	10	0
MNSS	150	10	40
MNSS	200	10	60
MNSS	300	5	100

Symptoms appearing in the rat were similar for both drugs and consisted chiefly in a depression of spontaneous activity and some increase in respiration. The depression of activity was noted principally with doses of 550 and 650 mg./kg. for N-123, and with 150, 200 and 300 mg./kg. doses of MNSS. Symptoms appeared 15 to 30 minutes after injection and continued for several hours after which there was a gradual recovery or a progression to death. No convulsions or hyperactivity were seen at any time. Occasionally there was a temporary paresis of the muscles of one forefoot, especially when the injected material extended to the region of the pectoral girdle. These symptoms were of the general order previously described by one of us[9] for other species, except that, particularly in rabbits, the depressive phase was preceded by some movement and agitation and apparent apprehension. These injections were intravenous, however, and in one case (rabbit 324) a convulsion occurred after 30 minutes, the animal dying 5 minutes later. It is of interest that the convulsion occurred during prostration.

With N-123, death occurred in rats in 12 to 70 hours (average = 30 hours), and with MNSS, in 18 to 140 hours (average = 48 hours). The autopsy findings were essentially the same for both drugs. The most constant findings were a bloody nasal discharge, bloody urine, small petechial hemorrhages in the lungs, and moderate enlargement and congestion of the spleen. With MNSS, the heart was frequently stopped in systole, and in these cases there was marked dilatation of both auricles and of the great veins. Microscopic appearances were the same for both drugs, namely, a few cases of marked degeneration of the lining cells of convoluted and collecting tubules of the *kidney* with accumulation of large amounts of protein material in the lumina of the tubules. In

one animal with N-123, the degeneration had progressed to a scattered necrosis of the tubular walls. Glomeruli and blood vessels appeared normal, and there were no significant changes in the liver or adrenals. At the site of injection there was usually moderate congestion of the blood vessels of the subcutaneous tissue but no necrosis.

Chronic Toxicity.—Rats were injected daily subcutaneously with varying doses of the two substances. These data (NU) are given in Table VII. Throughout the injection period, Groups VI, VII, VIII, and IX seemed somewhat more listless than normal. The animals of these groups all showed a loss of weight which was probably significant, especially when contrasted to the Control Group IV which gained in weight. On the contrary, the animals of Groups II and III, receiving only 5 mg./kg. of the two drugs, gained in weight to a greater extent than their control, Group I. While their gain in weight is significant, it is obviously not significantly greater than the gain in the control group, even though the gain of the control group is itself not statistically significant with certainty.

TABLE VII

EFFECT OF CHRONIC ADMINISTRATION ON BODY WEIGHT OF RATS

GROUP	SUBSTANCE	DAILY DOSE MG./KG.	N	NO. OF DOSES	MEAN BODY WEIGHT			P
					BEFORE	AFTER	PER CENT CHANGE	
I	Controls	–	10	–	217.9	237.9	+ 9.6	0.085
II	N-123	5	10	20	199.6	226.2	+13.3	0.037
III	MNSS	5	10	20	212.1	239.3	+12.8	0.013
IV	Controls	–	9	–	202.7	214.9	+ 6.0	
V	N-123	25	4	18	221.3	220.7	– 0.3	
VI	N-123	50	4	18	218.5	194.7	–10.9	
VII	N-123	100	4	18	230.0	189.0	–18.8	
VIII	MNSS	25	2	16*	209.0	194.5	– 7.0	
IX	MNSS	50	2	16*	208.0	186.5	–10.3	

*Injections not given on 6th and 7th days because of temporary lack of material.

In Table VIII the data (HLR) from a different series of experiments are given. These animals (mice, rats, and rabbits) were injected for pigmentation studies (see section entitled "Pigmentation"). Injections of N-123 only were made 5 days a week for 4 weeks. The results differed from the preceding in that the animals tolerated much higher daily doses; the 2 days' rest per week from the chemical may explain the difference. None of the mice or rats showed loss of weight up to 300 and 200 mg./kg. daily, respectively. Rabbits developed loss of weight at 200 mg./kg. In this table are also given data on three rabbits injected with methylnaphthoquinone. The loss of weight at a dose of 65 mg./kg. daily was not great, but the question of absorption is present since the material was suspended in polyvinyl alcohol. The dose of 65 mg. is equivalent on a molecular basis to 200 mg. of N-123. In another series of rats injected with N-123 for histopathologic studies all animals without exception gained in weight, the highest dose being 100 mg./kg. for ten doses.

In the series of rabbits injected for hematologic studies there was no significant loss of weight unless the dose was high enough to produce anemia, and even then the results were irregular. In rabbit 357, which received N-123 in 100 mg./kg. daily for 20 doses (5 doses weekly), there was no loss of weight and only a minor slowing up of the rate of growth, even though a moderate

anemia developed. In rabbit 350, which served as a control for 9 weeks and then received four 150 mg./kg. doses, there was a slight loss of weight which developed mostly after cessation of injections, and full recovery from anemia, the loss and recovery paralleling the changes in the red cell count. The anemia in this animal was severe, the red blood cells falling to 1.31 million.

TABLE VIII

PIGMENTATION TESTS WITH N-123 AND MeNQ

Five doses given per week for 4 weeks. Doses were subcutaneous except those starred* which were oral. All but the last three rabbits were treated with N-123.

SPECIES AND NO.	DOSE MG./KG.	INITIAL WEIGHT GRAMS	FINAL WEIGHT GRAMS	PER CENT CHANGE	PIGMENTATION
Mouse 1	100	23	24.7	+ 7	None.
Mouse 2	100	21	23.4	+11	None, died 5 weeks later.
Mouse 3	100	19	21.8	+15	One faint spot on back.
Mouse 4	200	17.5	25.6	+46	None.
Mouse 5	200	17	19.4	+14	None
Mouse 6	200	21.5	25†	+17	Around eyes, back of neck; killed after last injection.
Mouse 7	300	17	21	+24	None.
Mouse 8	300	20	24.7	+24	Scalp and forehead, forelegs, breast slightly spotted; color gone in 5 months.
Mouse 9	300	21.5	23.2†	+ 8	Numerous spots especially on abdomen; accidentally killed after last injection.
Rat 1	100	176	199	+13	Moderate: sides, head, forelegs.
Rat 2	100	169	190	+12	Slight: face, neck, sides.
Rat 3	100	192	205	+ 7	Slight: face, sides, head, perineal region.
Rat 4	200	183	219	+20	Strong: face, sides, sacrum, head, forelegs, perineal region.
Rat 5	200	195	226	+16	Strong: face, neck, abdomen, sacrum, head, all 4 legs.
Rat 6	200	163	195	+20	Strong: head, sacrum, perineal region, trace on sides.
		kg.	kg.		
Rabbit 407	100	2.20	2.62	+19	Slight after 1 week: sides, back and head.
Rabbit 408	100	2.27	2.82	+24	Slight after 1 week: sides.
Rabbit 409	50	2.20	2.67	+21	Slight after 1 week: sides and over eyes.
Rabbit 410	50	2.12	2.90	+37	Slight after 1 week: dorsum, trace on forehead.
Rabbit 368	100*	3.50	3.45†	- 1	None.
Rabbit 369	200*	4.62	3.69†	- 8	None.
Rabbit 412	300*	2.03	1.77	-13	None except margin of lips (contamination?).
The following tests were with MeNQ:					
Rabbit 411	32.5	2.14	2.67	+16	Trace after 1 week over haunches.
Rabbit 370	32.5*	4.20	4.22†	0	None.
Rabbit 371	65*	3.93	3.73†	- 5	None.

†Weight at 3 weeks.

Pigmentation.—The rats in groups VI, VII, VIII, and IX of Table VII developed a peculiar yellowish-brown pigmentation of the hair, which became evident toward the end of the injection period and deepened in the next one to three weeks after the injections were terminated. From then on there was no apparent change in intensity during 10 weeks of observation. The pigmentation, present chiefly in the hair over the face, back, and thorax, was scattered,

but sharply defined, and tended to be symmetrical. This pigmentation has previously been reported by us.[1] This phenomenon was observed in all rats on the higher doses of the two substances in both the Northwestern University and Hoffmann-La Roche laboratories. In addition, rabbits and mice in the latter laboratory were observed to develop the pigmentation (see Table VIII). All rats and nearly all rabbits with sufficient dosage developed it, but mice were less prone to show the color. The color was similar in the three species and always in discrete patches and nearly symmetrical. In all animals the color persisted for months, but finally disappeared. In one rabbit there was only slight fading after six months.

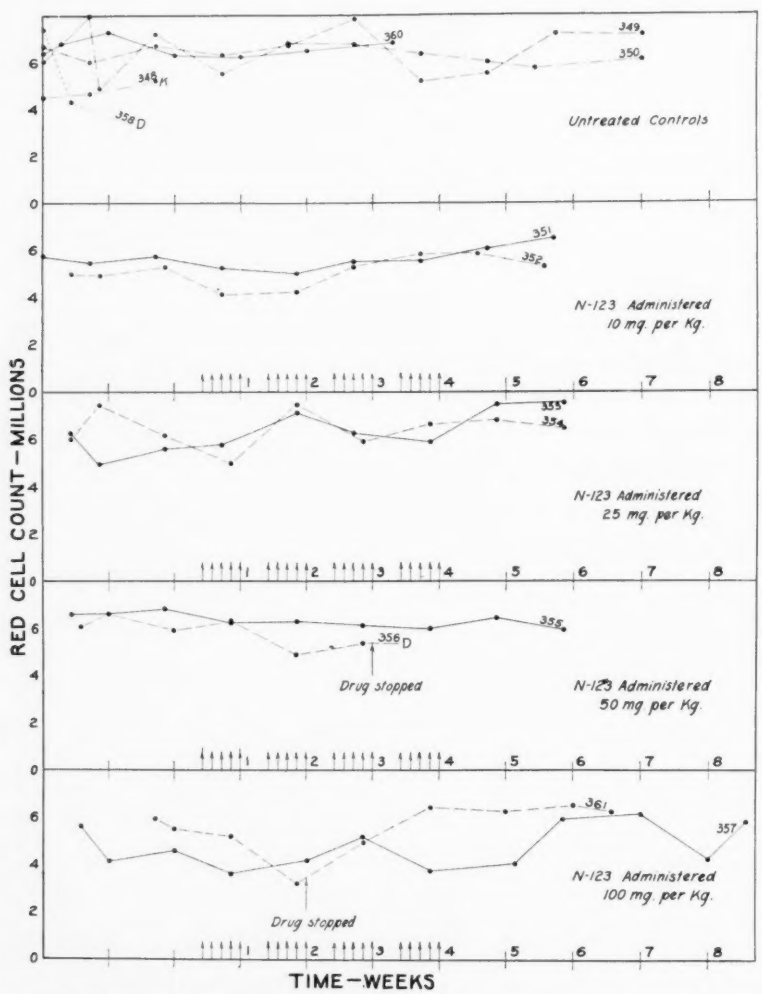

Fig. 2A.—Effect of repeated low doses of N-123 on the red cell count. Arrows indicate the daily injections. D = died.

When N-123 was given orally to rabbits, pigmentation did not occur. Methylnaphthoquinone administered subcutaneously in 3 per cent polyvinyl alcohol solution caused slight pigmentation in one of two rabbits which received 32.5 mg./kg. daily for 20 doses (during 4 weeks). This dose is equivalent to

100 mg./kg. of N-123. At twice the dose administered to one rabbit pigmentation did not occur. There is, of course, the question of absorption here. The MeNQ may possibly have been largely metabolized before absorption. Because MeNQ is irritating there occurred, at the site of nearly every injection, induration which persisted indefinitely, and later a few developed necrosis. The fact that coloring did develop in one rabbit indicates that the phenomenon has its origin in the methylnaphthoquinone nucleus itself, and that the phosphate or sulfonate gives greater effects only because of enhanced solubility. The mechanism of the color production can only be guessed at. A strong solution of N-123 applied daily to the hair had no effect. The color may be due to melanin formation directly or indirectly through metabolic changes in immature hair cells. Possibly vitamins are a factor. The symmetrical and discrete distribution suggests relationship to the color patterns of piebald animals or of nerve and vascular supply.

All rats were colorless after five months. The pigmented rabbits of this series cleared up somewhat faster than previous rabbits in the hematology experiment series.

Micropathology.—Sections from the livers, kidneys, and adrenal glands of the rats in Groups I, II, and III (Table VII) revealed no changes. The doses of N-123 and MNSS were small, 5 mg./kg.

In another experiment on rats (HLR), series of one, five, and ten injections of N-123 were given in 20 and 100 mg./kg. doses. Animals were killed the day after the single dose and three days after the last dose in the other two series. There were no significant changes in the livers or kidneys of any of these animals.

Some of the rabbits under hematologic study were examined. Two rabbits showed no significant gross or microscopic changes after N-123, given subcutaneously in 4 daily doses, 150 mg./kg. One of these animals (No. 349) was killed and autopsied when the red cell count was 1,570,000. The other rabbit (No. 364) was autopsied immediately after death following a convulsion. The convulsion was two days after the last injection, and the red cell count was 1,160,000 on the day of death. Two other rabbits died following similar doses, and delayed autopsy revealed little not attributable to post-mortem changes.

Hematology (HLR).—Eighteen rabbits were started, five serving as controls. Three of the latter were placed in the treated group after several weeks. Coccidiosis appeared in the colony, and one treated (No. 356) and one control animal (No. 358) died. Another control (No. 348) was killed because of severe diarrhea. Aside from these animals, coccidiosis was either mild or did not attack the other animals. N-123 was given daily in doses ranging from 10 to 200 mg./kg. Pronounced effects appeared with doses higher than 100 mg./kg., rabbits receiving the highest doses developing a severe aplastic anemia. Some died, and others recovered following withdrawal of the drug. One animal, No. 350, recovered even though the red blood cells dropped to 1.31 million on the third day after the 4th injection of 150 mg./kg. Following recovery the animals regained normal levels. The red cell counts taken weekly are illustrated in Figs. 2a and 2b. Rabbit 361 showed a drop in red blood cells to 2.90 million after ten 100 mg./kg. doses, but promptly recovered when injections

were stopped. Rabbit 357 actually showed a rise during the first three weeks of injections. Rabbit 360 was injected with 130 mg./kg. a week after the last of four 150 mg./kg. doses. As seen in Fig. 2b there was a temporary relapse after which recovery continued.

Observations were made also on white blood cells and hemoglobin. There were no significant changes except with the highest doses accompanied by severe anemia. In these animals there was usually a polymorphonuclear leucocytosis, but little effect was noted on the eosinophils, basophils, or monocytes. The white cell picture became normal with recovery from the anemia.

Effect on Blood Pressure and Respiration (NU).—Five dogs were used and were anesthetized with sodium pentobarbital. The carotid blood pressure was recorded on a kymograph tracing, and a simultaneous record of respiration was made by means of a pneumograph and tambour. All injections were made intravenously at a rate of 1 c.c. per 3 seconds.

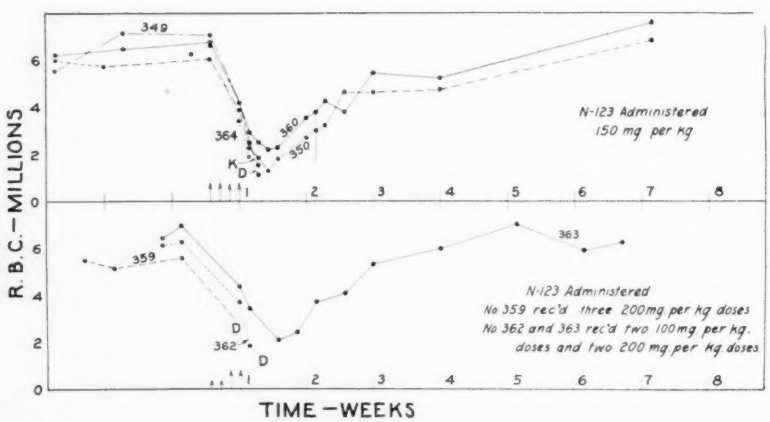

Fig. 2*B*.—Effect of repeated high doses of N-123 on the red cell count. Arrows indicate daily injections. Half-length arrows indicate a half-size dose in two of the rabbits. Total dose = 600 mg./kg. in all cases, except rabbit 360, which received an additional dose of 130 mg./kg., indicated by the vertical line at second week.

With doses of 20 mg./kg. of either substance, no significant alteration in blood pressure occurred. With 30 mg./kg. of N-123 an elevation in blood pressure began 1 to 2 minutes after the start of the injection, reaching a peak in about 4 minutes and declining gradually to normal in 5 to 10 minutes. No significant secondary increase or decrease occurred. The maximum elevation of arterial pressure was 40 mm. Hg. During this period there was a somewhat decreased respiratory rate. With MNSS 30 mg./kg. resulted in some cases in a transitory slight fall with increased depth of respiration and in other cases no significant changes in either arterial pressure or respiration.

Forty mg./kg. of N-123 caused in one dog a rise in blood pressure of 70 mm. Hg and a greatly increased respiratory rate which was accompanied by a violent stirring about of the animal and an awakening from anesthesia. After 15 seconds, during which time there was no abatement of symptoms, a deeper anesthesia was induced and recovery followed. Similar effects were obtained in cats[9] with 10 mg./kg. doses of N-123 under ether or alurate anesthesia, but the blood pressure reaction was somewhat less under ether. N-123

had little effect on respiration in morphinized rabbits until high doses were given, in which case respiration was depressed.

SUMMARY

1. Two compounds, tetrasodium 2-methyl-1, 4-naphthohydroquinone diphosphoric acid ester $\cdot 6H_2O$ (N-123) and 2-methyl-1, 4-naphthohydroquinone sodium bisulphite complex (MNSS) were studied for vitamin K activity and pharmacologic effects.

2. In bile duct obstructed rats or rats made deficient in prothrombin by dietary means (30 per cent petrolagar in powdered Purina dog chow) 2 to 4 gamma per rat caused a significant shortening of the prothrombin clotting time (Quick method). In one series, 2 gamma of either substance caused the prothrombin time to return to normal and ¼ gamma appeared to be the threshold dose.

3. After the prothrombin time is brought back to normal by the administration of the therapy (about 8 gamma) and then the therapy is stopped, the prothrombin time returns practically to the deficiency level in 5 days.

4. It was shown that vitamin K does not affect prothrombin time on being added to the blood of patients or rats with a prothrombin deficiency.

5. Weight for weight MNSS appeared to be slightly more active, but on a molecular basis N-123 was about 40 per cent more potent.

6. The higher molecular activity of the diphosphate than either MNSS or methylnaphthoquinone is not due to any direct action on the blood in vitro.

7. The LD50 in rats for N-123 is 610 mg./kg. and for MNSS is 175 mg./kg. by subcutaneous injection.

8. In rats receiving high doses of either drug, there was a lowering of spontaneous activity and an increase in respiration.

9. In dogs under pentobarbital anesthesia doses of 30 to 40 mg./kg. caused a marked rise in pressure and respiratory stimulation. MNSS did not exhibit these effects.

10. Lethal doses with both drugs administered to rats resulted in gross and microscopic changes, especially general petechial hemorrhages and renal degeneration.

11. The chronic administration of 100 mg./kg. of N-123 in rats resulted in no pathologic changes.

12. In rabbits, doses of N-123 above 100 mg./kg. caused an "aplastic" anemia from which complete recovery occurred in some animals; others died. Those dying showed no significant microscopic changes directly attributable to the substance. Except for a transient polymorphonuclear leucocytosis during the aplastic anemia there were no significant changes in animals recovering.

13. Mice, rabbits, and rats all developed a yellow to orange-brown pigmentation of the fur in from one to three weeks following a series of medium to large doses of either substance. This pigmentation remained for months with but slow fading. Mice were the least susceptible to the pigmentation phenomenon.

14. The margin of safety between the therapeutically effective and toxic dose of the two preparations is relatively enormous.

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THE EFFECTS OF VITAMIN B DEPRIVATION ON SPONTANEOUS ACTIVITY OF THE RAT*

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THE effect of vitamin deficiency on activity is not only of biologic interest, but in time of war, when food supplies are short, it is also a matter of great practical moment. There is a general impression among clinicians that deficiency of the Vitamin B complex results in fatigue and sluggishness both mental and physical; this impression has recently been supported by certain purposeful studies. Williams and his associates in 1940 found that women placed on a diet extremely low in thiamine developed inactivity and apathy in addition to loss of weight and prostration. In a later study (1942), in which the thiamine deprivation was less extreme, they also found "physical inefficiency." There were no quantitative measurements. O'Shea, Elsom, and Higbe (1942), however, detected no deterioration in the speed of hand muscle coordination when subjects were deficient in the B vitamins and no improve-

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ment after therapy with Vitamin B. Williams and Mason (1942) produced no noticeable symptoms in people placed for six months on a diet supposed to be deficient in riboflavin. Simonson, Baer, and Enzer (1942) made the interesting observation that excessive intake of the Vitamin B complex did not affect ability to perform five different types of muscular work, but the treated subjects felt a subjective improvement of working capacity. Finally Keys and Henschel (1942) showed that additions of vitamins to the standard ration did not improve the physical performance of men in the army.

From the laboratory standpoint Guerrant and Dutcher (1940) found that young rats (23 to 26 days old) placed on a diet deficient in the B complex but supplemented with riboflavin and a rice polish concentrate, so that the food was presumably deficient only in thiamine, were more active for a period of three to four weeks than controls which received thiamine supplements. As the effects of vitamin depletion became severe, activity fell off but increased again when Vitamin B_1 was added to the diet.

Because of the indefinite, and indeed contradictory, character of some of the results in the above reports, it seemed useful to attempt further measurements of the spontaneous activity of rats on deficient diets. The present paper deals with this problem.

METHODS

There is a huge literature on measurement of the activity of the rat under various conditions. Briefly, for our present purpose, it may be said that female white rats, when placed in suitable "running" or revolving cages, display a variable amount of spontaneous activity. This depends upon many conditions: the stage of the estrus cycle, season, temperature, noise, diet, and other factors. Some rats may not run at all; others cover surprising distances in 24 hours, in some cases as many as twenty to thirty kilometers. Most rats when placed in the revolving cages are sluggish at first and then gradually increase their activity until a more or less constant level of daily running is achieved. All these matters have received detailed attention on the part of previous investigators (Slonaker, 1912; Shirley, 1928).

The type of cage used in our experiments (Slonaker, 1908) is a wire mesh cylinder with a circumference of 1.5 meters, which rotates about an axle with very little friction. Each revolution is recorded by a cyclometer on which the total can be read at any time. Within the revolving cage is a smaller stationary box, with food compartment, in which the animal reposes when not running. A battery of twenty such cages were set up in a large room, which for the most part was quiet and had a fairly uniform temperature. The animals were weighed daily, and during the experiments the quantity of food eaten was measured or controlled.

EXPERIMENTS

Experiment 1.—Twenty young female rats, weighing from 80 to 100 grams, were separated into a group of 10 test animals and 10 litter mate controls. Each rat was placed in one of the running cages and was allowed to eat as much of the stock diet* of the laboratory as it desired. The animals and the food were weighed each day and the number of revolutions run was recorded.

*Casein 16, cornstarch 44, yeast 10, salt mixture 4, cod liver oil 10, alfalfa 2 and lard 14.

After a control period of three weeks, when the level of activity seemed stabilized, the ten test rats were changed to a diet deficient in Vitamin B complex. This consisted of washed "vitamin-free" casein 17, cornstarch 64, cod liver oil 10, Crisco 5, and salt mixture 4. On this diet, even though unrestricted in amount, rats soon begin to lose weight; whether because of lack of appetite or because of some specific effect of vitamin deficiency is not certain. The ten control animals continued to receive the stock diet, but the quantity was limited so that they lost weight at about the same rate as the rats on the deficient diet. Thus they served to control any nonspecific effects of inanition. Aside from loss of weight all of the animals appeared well throughout the experiment.

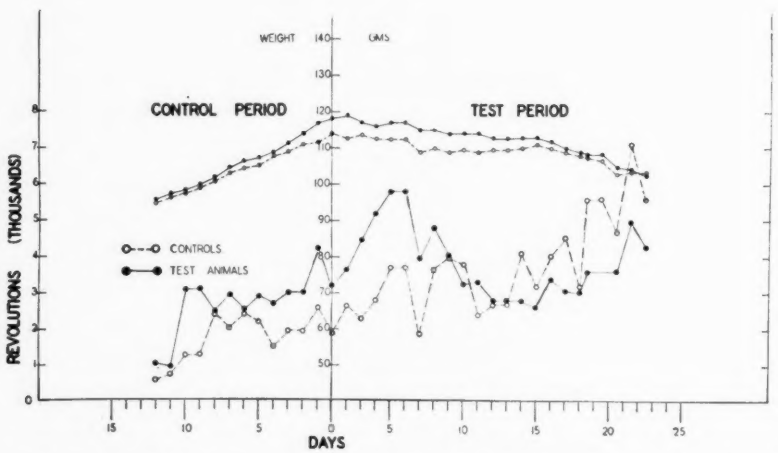

Fig. 1.—Summary of Experiment 1. The first portion of the control period is not shown.

Results of Experiment 1.—The first point to be noted is the great individual variations in activity. These seem important and will be discussed below, but we will present first a composite picture of the test and control animals. Fig. 1 gives the results. The average number of revolutions run daily by the rats in the two groups is shown along with the average weights. The test animals, during the last ten days of the control period, had reached an approximate level of activity in the vicinity of 3000 revolutions. From the start of the test period there is an increase in activity of both sets of rats. This increase, however, is much more marked with the animals on the deficient diet, and within a few days activity is double the control level. The difference in the two groups is statistically significant. After reaching a peak at the fifth day, activity of the test animals falls off rapidly, and within 10 days is back to the control level. A few days later there is again an increase in activity, which coincides with rapid weight loss to a point where the animals' lives are in danger.

In contrast to the test animals, the controls show an entirely different pattern. There is to be sure a slight initial gain in activity, but there is never any drop; from the 15th day on, coincident with rapid weight loss from food restriction, there is a great increase in activity which surpasses that shown by the test animals.

The weight curves require a separate word. During the control period there is the usual brisk gain, almost in a straight line, which occurs with rats of this age on the stock diet of the laboratory. Change to the Vitamin B deficient diet is followed by an immediate slackening in the rate of gain and within a day or two by weight loss, gradual at first, but after approximately two weeks more rapid. The sharp spurt of activity and the subsequent fall are not related to any change in type of the curve of weight loss.

Discussion.—Statistical analysis of the curves of Experiment 1 indicates that the following conclusions may be reliably drawn: (1) Vitamin B deprivation and/or limitation of food intake increase activity initially; (2) limitation of the amount of the normal ration causes progressive rise in activity; (3) vitamin B deficiency after the initial phase of stimulation results in a progressive depression relative to the activity seen in the controls, so that the final (premortal?) stimulation is much less than is to be expected from comparison with the controls in the same state of inanition.

The cause of the increased activity of the rats on the Vitamin B deficient diet is not clear. Guerrant and Dutcher (1940) suggest nervousness from vitamin deprivation as one explanation. However, their young rats which had not reached a steady level of activity before the experiment was started showed increased activity for a period of weeks, an entirely different situation from our experiment in which older rats at a level of activity show only a brief spurt after vitamin deprivation. In other words, a fall in activity in our animals long before serious signs of deficiency are present cannot be well explained on this theory. Another possibility is that change to an inadequate diet which may be distasteful stimulates increased activity in the search for more palatable food.

One can hardly draw any practical applications for man from these experiments, but they suggest that a brief deprivation of Vitamin B may not lessen spontaneous activity, but rather the contrary. It also appears that in rats, at least, restriction of the total amount of food, as in our controls, provided additional vitamins are not fed, tends to increase spontaneous activity slowly over a period of at least three weeks. The final answer to the cause of this phenomenon is not at hand. It is suggestive, however, that in the controls of Experiment 2, to be presented below, where an adequate Vitamin B intake was assured by feeding it to each rat, the same limitation of food did not result in a progressive rise in activity. Hence it is possible that the slow increase in running in the control group in Experiment 1 was the expression of a slowly developing B deficiency brought about by the limitation of food intake and with it of available Vitamin B.

The Results in Individual Rats.—As indicated above, there were striking variations in the activity of individual rats both in the test and in the control series. Among the test animals, for example, No. 6 did not run at all during the entire experiment (see Fig. 2*A*). The cyclometer stood at zero not only during the control period on stock diet but after the Vitamin B deficient diet was instituted. There was no "premortal" increase of activity in spite of very rapid weight loss. The theories discussed above in connection with the composite curve (Fig. 1) all break down as explanations of this animal's

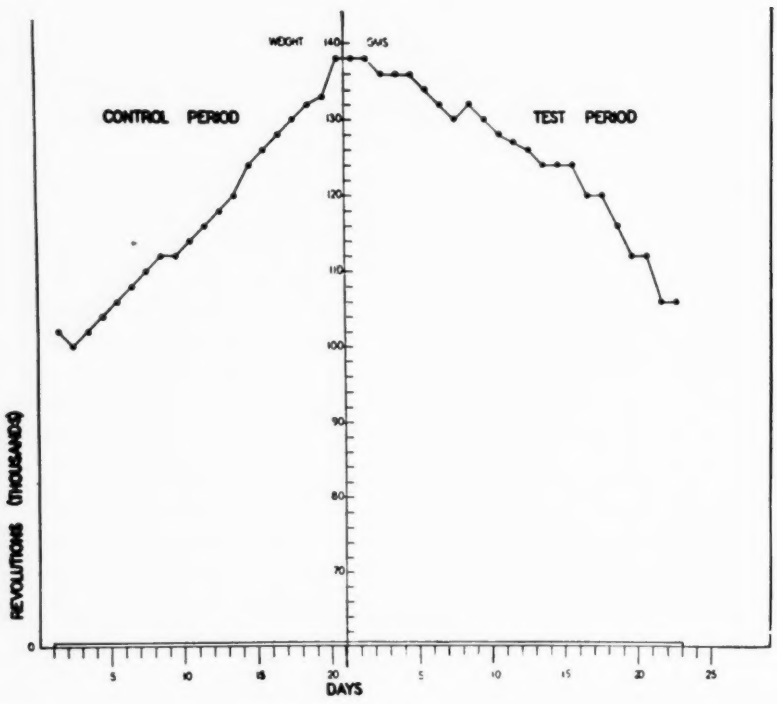

Fig. 2A.

Fig. 2 (*A*, *B*, *C*, *D*, *E*).—Weight curves and activity of individual rats of Experiment 1. See text.

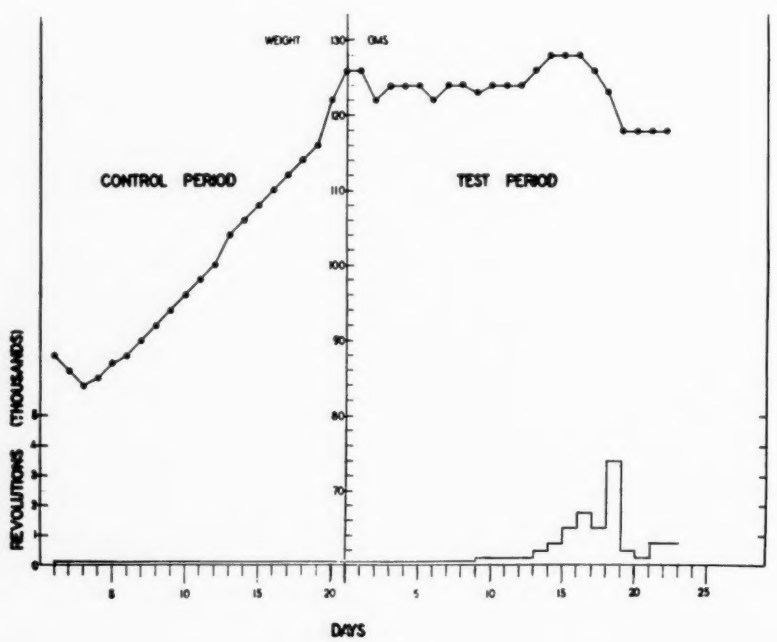

Fig. 2B.

behavior. Rats 5, 4, 2, and 8 of the test series failed to run during the control period and during the early days of the deficiency period, but finally showed slight activity beginning respectively on the seventh, ninth, thirteenth and fifteenth days. There was no correlation, however, between the degree of such activity and the fall in the weight curve (see Figs. 2*B* and 2*C*). Five animals of the test series (Nos. 1, 3, 7, 9, and 10) began to run after the start of the experiment, and by the end of the control period were very active, although the pattern varied in different rats. In No. 7 for example (see Fig. 2*D*) activity was still rapidly increasing at the end of the control period. This rise continued sharply for 6 days; there was then a slight fall with a secondary marked rise even though the animal was not losing weight rapidly at that time. No. 3 (see Fig. 2*E*) failed to show any definite terminal rise in activity even though it was losing weight rapidly.

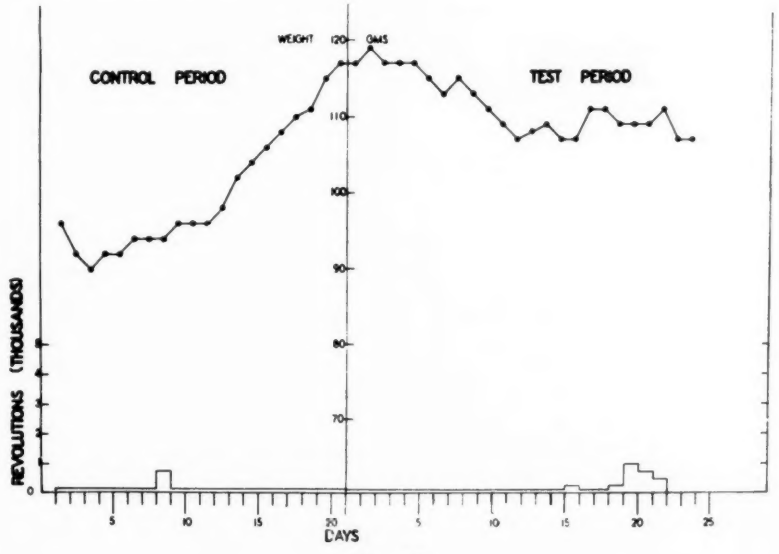

Fig. 2*C*.

Among the control series there were also various types of reaction. One animal failed to run throughout the whole experiment. But whether or not the animals ran during the control period, activity during the test period always increased, even if only slightly, and never showed the invariable spurt of increased activity followed by decline exhibited by the test animals.

Experiment 2.—Because of the possibility that in Experiment 1 the factor responsible for the changes was the alteration in food, rather than the lack of Vitamin B, a second experiment was run in which the food remained unchanged throughout for both groups of rats, and Vitamin B was administered separately. During the control period both test and control animals were placed on the Vitamin B deficient ration described above, but in addition each received 1 c.c. daily of a Vitamin B concentrate which contained according to the manufacturer's statement, in 30 c.c., Vitamin B_1 1500 International Units, riboflavin 1.2 mg., vitamin B_6 4.5 mg., pantothenic acid 12.0 mg., nicotinic acid 60.0 mg. It was also said to contain the filtrate factor (Jukes-Lepkowsky

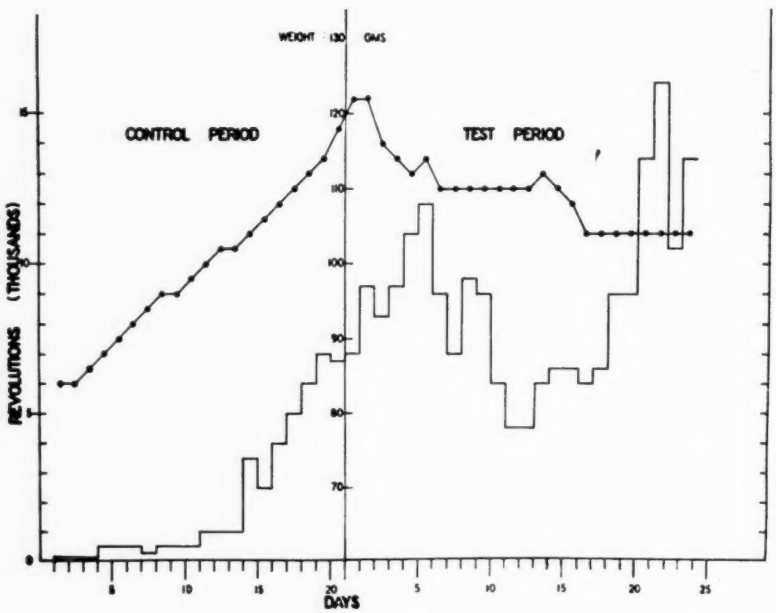
WEIGHT GMS
CONTROL PERIOD
TEST PERIOD
REVOLUTIONS (THOUSANDS)
DAYS

Fig. 2*D*.

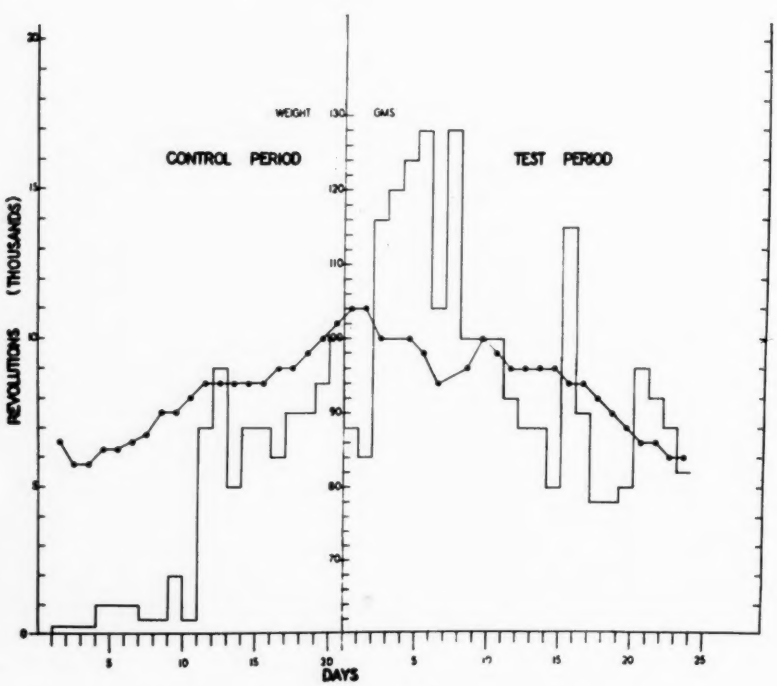
WEIGHT GMS
CONTROL PERIOD
TEST PERIOD
REVOLUTIONS (THOUSANDS)
DAYS

Fig. 2*E*.

value of 28 per c.c.).* This vitamin concentrate was fed separately to each rat, mixed with a few drops of water. At the end of the second control period of 21 days the Vitamin B supplement was withdrawn from the test rats. As these rats lost weight, the quantity of food allowed to the controls was restricted so that they too lost at approximately the same rate, but they continued to receive the full dose of B concentrate. During the control period of 21 days the average gain in weight of the rats in Experiment 2 was 18 Gm. This is definitely less than the gain made by the rats in Experiment 1, which averaged 23 Gm. during the comparable period. We interpret this to mean that the B deficient diet plus B concentrate was not as adequate a ration as the stock diet used in Experiment 1.

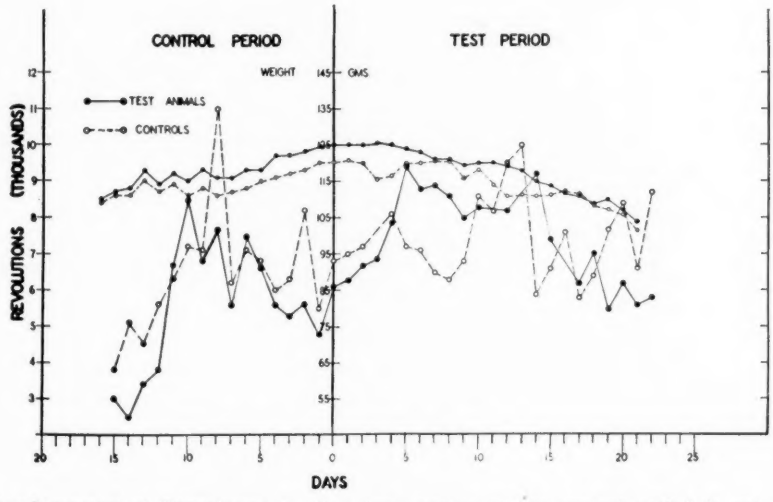

Fig. 3.—Summary of Experiment 2. The first portion of the control period is not shown.

The results (see Fig. 3) are, however, in essential agreement with the first experiment. The animals deprived of the vitamin supplement promptly increased their activity to approximately double the control level; in this case the high level was maintained for a longer period than in Experiment 1. However, there was subsequently a fall to the control level. At this point the experiment was modified by restoring the B concentrate to the test group; the results of this procedure will be discussed later. The control group of rats, although showing considerable irregularity from day to day, did not undergo any consistent deviation from their control level. A statistical analysis of the curves demonstrated that the following conclusions were justified:

1. Vitamin B deprivation was followed by a slightly greater increase of activity than deprivation of food alone. The mathematical chances are 18 to 1 in favor of this being a true difference.

2. After the initial phase of stimulation, Vitamin B deprivation produces a progressive depression of activity as compared to the controls whose food intake is limited without vitamin restriction.

*This was a specially fortified product supplied for this study by the Galen Co., Inc., Berkeley, Calif.

After three weeks both sets of animals were restored to the original diet, that is, they all received the B-free diet as desired plus the 1 c.c. of Vitamin B concentrate just as during the control period. The effect on activity is illustrated in Fig. 4. The six days preceding restoration to the original diet are shown, and it is seen that the activity of the test rats was practically stationary, whereas that of the controls was increasing. On the change to the original diet both sets show an immediate drop in activity to a very low level, which during the ensuing days increases gradually and slightly. The meaning of this interesting finding is not clear. Sudden restoration of vitamins which had been lacking does not suffice as an explanation, because the control group, although restricted in quantity of food, had been receiving the full amount of Vitamin B throughout the experiment. It appears that the correction of either lack of Vitamin B or lack of total calories leads to a decrease of activity, which may be due to release of nervous tension or cessation of need for seeking food. It may simply be that the rats, whose food intake approximately doubled during the first few days of unrestricted diet, were busy eating and therefore ran less, or that the unusual distention of their gastrointestinal tracts caused a disinclination to exercise.

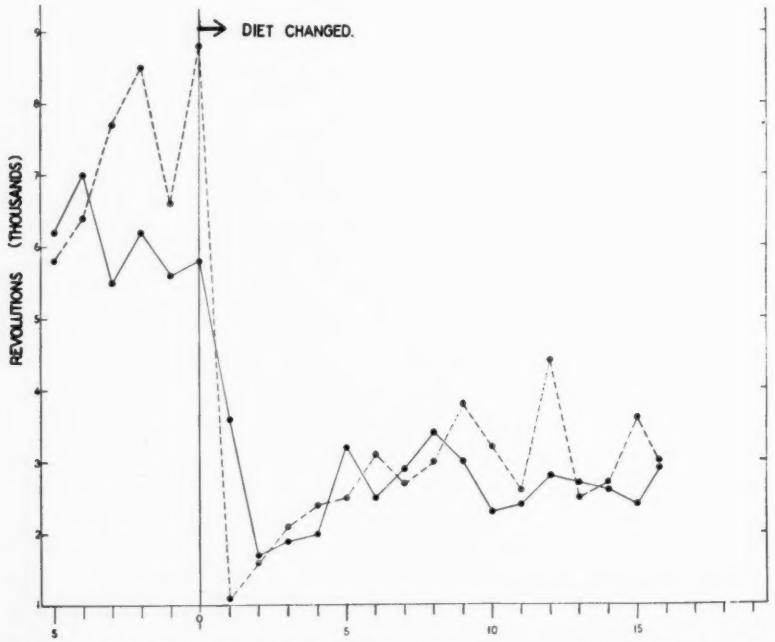

Fig. 4.—Effect on activity of restoration of diet to the conditions obtaining during the control period of Experiment 2.

DISCUSSION AND SUMMARY

To summarize briefly, the object of these experiments was to discover the effect of Vitamin B deficiency on spontaneous activity of the rat. Two experiments along somewhat different lines were done so as to be sure of adequate controls. It was found, in agreement with previous observers, that rats placed in revolving cages do not run at first; they seem to have to learn. In our

observations there was an increase of activity during approximately the first two weeks of the control period; thereafter activity remained more or less on a level. There were great variations, however, in individual animals; some did not run at all during the entire experiment; others became active promptly; some were still increasing their activity at the end of the three weeks' control period.

In any event withdrawal of Vitamin B from the diet was followed by a prompt spurt of activity lasting from five to ten days and then followed by a decline to approximately the control level. This phenomenon occurred with great regularity and was strikingly different from the behavior of the control animals whose food intake was curtailed. These rats showed a slower but continued increase of activity, which went on, with no drop, throughout the course of the experiment, unless a special supplement of Vitamin B was administered.

The explanation of the sharp spurt of activity shortly after vitamin deprivation is not clear. The suggestion of Guerrant and Dutcher that B deficiency causes "nervousness" does not seem adequate to us: first, because the effect is so very prompt, and, second, because there is no evidence that nervousness in the usual sense of the word gives rise to increased running in this type of experiment. During the period of Vitamin B deprivation our rats were very restless, excitable, and difficult to handle. However, this variety of "nervousness" does not necessarily result in increased running, as has been amply demonstrated by one of us (T.) in studies to be reported later. The running response probably requires a conscious purposeful co-ordinated "drive," which is quite different from the lack of neuromuscular poise or restlessness which is usually called "nervousness." The simplest view would seem to be that shortage of either vitamin or food stimulated the animals into a reaction pattern which would be helpful in the search for a more desirable diet. The drop in activity of the vitamin deficient rats following the initial stimulation seems on the other hand specifically related to Vitamin B lack since it does not occur in the controls. It should be noted that this slowing of activity occurred in our animals long before they showed any visible evidence of deficiency disease; it cannot be regarded at all as an agonal phenomenon.

The sudden drop of activity of rats when either Vitamin B or unlimited calories is restored after a previous period of limitation is also of great interest (see Fig. 4). The only satisfactory explanation is that the fulfillment of a dietary need of any sort is followed by greater food intake, more time spent in feeding, and subsequently in sleeping, and the cessation of the urge to run associated with unsatisfied cravings. This is probably similar to the satiation and tendency to inactivity commonly experienced after an unusually large meal.

Finally, one is tempted to speculate on the bearing of these findings on problems of human nutrition. The suggestion comes forth that in situations where only a small bulk of food can be available, such as in the case of parachute troops or in castaway rations, the supplying of Vitamin B might be just as important over periods of a few days as adequate calories. Our experiments also suggest that Vitamin B deficient diets can cause alterations in

behavior which may precede by many days any other clinical evidence of the vitamin deficiency and may in fact be the only symptom of such deficiency in the subclinical stages.

CONCLUSIONS

1. Limitation of Vitamin B in the diet of the white rat is promptly followed by increase in activity.

2. After a period of a few days to two weeks there is a sharp decrease in activity.

3. This decrease is not to be explained by inanition, since control fasted rats whose Vitamin B intake is maintained at a constant level continue to show normal activity.

4. When rats whose diet has been deficient in either Vitamin B or in total calories are furnished unlimited food and adequate vitamins, there follows, not an increase, but a sudden sharp fall in activity, which persists during the period of increased food intake.

5. Some possible explanations of these phenomena are discussed.

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THE REACTION OF LEUCEMIC PATIENTS TO THE SULFONAMIDES

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OUR knowledge of the effectiveness of the sulfonamides on certain infectious diseases is rapidly becoming definite and accurate. Our knowledge of the pharmacologic action of the several forms is still accumulating. The earliest report[1] indicated that the drugs produced a bacteriostasis. The suggestion of the neutralization of the bacterial toxins[2] could only be a partial explanation. Very early it was learned that the temperature greatly affected the activity of the drug. The fact was still left unexplained why certain bacteria were either prevented from multiplying or were killed outright, while other bacteria were seemingly unaffected. Observations of later date suggest the importance played by the medium.[3, 4] It would seem rather certain at the present time that the sulfonamides may destroy certain substances necessary for bacterial multiplication,[5-7] and it is with this theory we are chiefly concerned.

Much experimental evidence has accumulated concerning the toxic effects of the drug. For our purposes only that effect on the white blood cells will be considered. Young[8] apparently was one of the first to report complete agranulocytosis. Many others have demonstrated this change and have offered several explanations. The conclusion of Bliss and Long,[1] that with the exception of toxic changes associated with malignant neutropenia there was no direct effect upon the white cells of the patient, seems adequate. It should be noted that the severe cases of neutropenia usually occur only after the drug has been administered for some time, generally from two to four weeks. Goodman and Gilman[9] suggest that this reaction is dependent upon the factor of personal idiosyncrasy, adding that the reaction is rare. Attention should be called to the fact that when an infection is accompanied by a leucopenia, the administration of the drug may result in a rapid rise in the number of granular white cells.

It remained for Livingston and Moore[10] to demonstrate the effect of sulfapyridine on the white blood cells of patients with leucemia. They administered sulfapyridine to six cases of lymphatic leucemia and reported a definite and marked change in the total number of lymphocytes in the peripheral blood. The first patient with chronic lymphatic leucemia was treated on four separate occasions. The drop in white cells was: from 256,000 to 6,500; from 33,000 to 11,000; from 47,000 to less than 20,000; and from 94,000 to slightly over 20,000. Although this patient was treated late in 1939, the outcome was not stated. Their second patient who apparently developed signs and symptoms of acute lymphatic leucemia showed a drop in the total white count from 212,000 in successive stages of: 183,000 to 100,000 to 60,000 to 40,000 and finally to 20,000. There was no stated evidence of any clinical improvement, and they state that

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the drug was discontinued on the 19th day because "in spite of the change in blood picture there was clinical evidence of widespread leucemic infiltration."

Following the lead suggested by Livingston and Moore four cases of lymphatic leucemia were studied, and the results tend to agree with those found by the previous investigators.

CASE 1.—Mrs. O. B., 36-year-old, white, was admitted May 4, 1941, complaining of general weakness, edema of the legs, and sore throat of one-week duration. Several small lymph nodes were found in the neck and groin. The spleen was only slightly enlarged. Her blood count showed: hemoglobin, 55 per cent; red cells, 2,520,000; and white count as given below. Several small macular areas and small ecchymotic areas were noted on her legs. In Table I are listed her blood counts with her therapy and progress:

TABLE I

DATE	WHITE CELLS	LYMPHOCYTES	REMARKS
4/ 7	89,000	98	Transfused with 400 c.c. citrated blood.
4/ 9	74,900	98	
4/10			Sulfanilamide started, 1 Gm. every four hours.
4/12	8,350	99	
4/12	5,000	98	
4/13	6,200	95	Sulfanilamide stopped. Transfused 600 c.c. citrated blood.
4/14	13,600	98	
4/15	29,200		
4/16	22,000		
4/17	41,800		Sulfathiazole started 1 Gm. every four hours. Patient irrational.
4/18	20,700		
4/19	5,500	97	
4/21	4,200		Sulfathiazole stopped. Patient very confused.
4/22	2,900		
4/23	1,450		
4/24	2,700	80	Patient irrational.
4/25	2,150		
4/26	2,100		
4/27	1,600	82	
4/28	2,700		
4/29	2,300	69	Condition improved. Temperature normal for first time in hospital.
4/30	3,200		
5/ 1	3,100		
5/ 2	4,200	66	
5/ 5	5,500	36	Neutrophiles, 64 per cent.
5/ 7			Patient up on recliner.
5/ 8	5,400	43	Neutrophiles, 57 per cent.
5/11	7,200	56	Neutrophiles, 44 per cent. Discharged from hospital.

The patient left the hospital with a blood picture of: hemoglobin, 58 per cent; red cells, 2,860,000; and a white count as given above. She received no more of the sulfonamides.

Twenty-seven days after her discharge she was admitted to another hospital. On admission her white count was 32,000 with 97 per cent lymphocytes. On June 14, ten days after admission, with her white count 16,900, she was given sulfathiazole in 1 Gm. doses every four hours. The following day her white count dropped to 3,600, and four days later it was 400, dropping to 300 on the following day. Eight days after sulfathiazole was begun the patient died. Post-mortem examination revealed atrophic bone marrow and splenomegaly. The anatomic diagnosis was acute lymphatic leucemia.

CASE 2.—J. D., a 76-year-old white male, was admitted Sept. 24, 1941, complaining of fatigue, dyspnea, palpitation, and precordial discomfort. His blood count on admission was: hemoglobin, 26 per cent; red cells, 904,000; white count, 306,000; 96 per cent lymphocytes. In Table II are listed his blood counts with his therapy and progress.

TABLE II

DATE	WHITE CELLS	LYMPHOCYTES	REMARKS
9/24	306,000	99	
9/25	576,000	100	Sulfathiazole started 1½ Gm. every four hours for two doses. Pulse weak and thready, respirations rapid and labored, transfused with 750 c.c. blood.
9/26	450,000	99	
9/27			Sulfathiazole started ½ Gm. t.i.d.
9/30	297,600	98	
10/ 2	409,000	99	Patient died.

This patient showed symptoms of vascular collapse only one hour after the second dose of sulfathiazole was given and received only ½ Gm. three times a day after the 27th.

Case 3.—E. P. (H. H.), a 39-year-old white female, for the preceding two weeks had suffered from canker sores in mouth, dysuria, and frequency. A blood count taken during this time showed a hemoglobin of 50 per cent; red cells, 2,400,000; white cells, 6,000 with 24 per cent polys and 76 per cent lymphocytes. She developed abdominal distention and progressive weakness and was admitted to the hospital. Her physical examination on admission showed no enlargement of the spleen or liver, and no enlarged lymph nodes were found. In Table III are listed her blood counts with therapy and progress:

TABLE III

DATE	WHITE CELLS	LYMPHOCYTES	REMARKS
9/29	34,000	90	
10/ 1	46,350	82	Transfused 400 c.c. whole blood.
10/ 3	47,400	90	Sulfathiazole 1 Gm. t.i.d.
10/ 5	37,000	90	
10/ 6	31,000	94	
10/ 7	29,700	93	Patient felt fine, temperature normal for past 24 hours. Sulfathiazole stopped, total of 9 Gm.
10/ 8	23,800	92	
10/10	20,150	86	
10/11	17,550	88	
10/12	18,350	87	
10/14	14,300	72	During past five days has improved, temp. normal.
10/16	42,250	70	Sudden rise in temperature—104° F.
10/17	48,050		Sulfathiazole started, 1 Gm. t.i.d.
10/18			Temperature lower, condition same.
10/20			Patient died.

The patient developed a terminal pneumonia. A post mortem was performed; the diagnosis was acute leucemia.

CASE 4.—H. D. W., a 68-year-old white male, was admitted March 8, 1942, in extremis. About two months previous to admission the patient first noticed a mass in the left axilla which had increased in size. Two weeks previous to admission he noticed several masses in the left side of the neck. These masses also had progressively increased in size. Physical examination revealed enlargement of the liver and spleen. Discrete, nontender, and moderately firm lymph nodes were found in both sides of the neck, axillae, groin, and popliteal spaces. In Table IV are listed his blood counts with his treatment and progress. The temperature which was at 103° dropped progressively after sulfanilamide was given to 100°. The report of the biopsy was acute leucemia, type not determined. On the 18th the patient was well enough to be up and about. His appetite improved as well as his general condition and he was allowed to go home.

The patient was readmitted on May 29, 1942, with a semifluctuant mass on the anterior surface of his right leg, of ten days' duration. He had been well enough to return to his former job as janitor, until ten days previously when he developed "flu." He was referred to the hospital for treatment of the leg. Physical examination revealed large lymph nodes

TABLE IV

TEMP.	DATE	WHITE CELLS	HEMOGLOBIN PER CENT	LYMPHOCYTES PER CENT	RED CELLS	REMARKS
102	3/ 8	201,000	42	99	1,920	Patient very drowsy
	3/ 8	271,000		100		
102.6	3/ 9	303,000	49	99	2,287	
102.8	3/10	213,600	49	99	2,136	Sulfanilamide started, 1 Gm. every 4 hr.
101	3/11	220,000	49	99	2,290	Refuses to eat.
100.6	3/12	170,000	49	99	2,349	Sulfanilamide stopped.
100	3/13	102,400	42	97	1,967	Neck glands dec. markedly.
100.6	3/14	154,600	42	99	2,175	Transfused 500 c.c. whole blood.
98	3/16	204,000	45	97	2,406	Biopsy of node in neck.
100	3/18	240,600	39	98	2,580	Sulfadiazine started, 135 grains in first 24 hours, then 2 Gm. every 4 hr.
99.6	3/20	311,200	49	99	2,928	
99.2	3/23	344,000	49	99	2,486	Sulfadiazine stopped, sulfathiazole started, 135 grains in first 24 hours, total 225 Gm.
98.6	3/25	396,600	39	99	2,306	
98.6	3/27	303,500	45	99	2,077	
98.6	3/28					Discharged.

TABLE V

TEMP.	DATE	WHITE CELLS	HEMOGLOBIN	LYMPHOCYTES	RED CELLS	REMARKS
101	5/30	431,000	32	99	1,519	Patient very drowsy. No appetite.
102	5/31	384,000	36	100		Sulfanilamide started, 1 Gm. every 4 hr.
101.7	6/ 1	300,000		100		
100.4	6/ 2	186,000		99		
100.4	6/ 3	165,000	29	100	1,450	Sulfanilamide stopped.
101	6/ 4	202,000		99		
101	6/ 6	194,000				Sulfanilamide started, 1 Gm. every 4 hr.
100.2	6/ 7	265,000				
102	6/ 8	266,000				
100.4	6/ 9	278,000				
101	6/10	170,000				
100	6/11	192,000				Patient says he feels much stronger.
100	6/12	159,000				Up on recliner.
100.8	6/13	119,000				Sulfanilamide stopped.
101.8	6/15	104,500	32	98	1,655	
100	6/16					Discharged.

throughout body as well as enlarged liver and spleen. The mass on the leg became definitely fluctuant and was incised and drained five days after admission. In Table V are listed his blood counts with his treatment and progress.

The patient ran a septic temperature ranging from 100.4° to 102° for the first four days, after which it ranged between 97° and 101.° The glands in the neck decreased in size during his stay in the hospital.

After the patient's discharge from the hospital, the lymph nodes again enlarged and he grew progressively worse, dying in July of the same year. He did not receive any of the sulfonamides after leaving the hospital.

DISCUSSION

The first patient, suffering from acute lymphatic leucemia, reacted promptly to the administration of sulfanilamide and sulfathiazole by a drop in the total

white cells, followed by clinical improvement from a moribund state to that of comparative health. Contrary to prevailing teachings, the lymphocytes were decreased while the number of neutrophiles remained nearly constant. Because of the prolonged leucopenia, it was thought best to discontinue the sulfonamides.

Patient 2 showed the smallest hematologic change after the administration of sulfathiazole. He received only six Gm. over a four-day period. The sudden vascular collapse after the administration of sulfathiazole is difficult to explain.

The third patient like the first had the acute form of the disease and rallied from a nearly moribund state after sulfathiazole was given. The clinical improvement was marked. It is quite apparent that the drug should have been continued.

The fourth patient, suffering from acute lymphatic leucemia, was in extremis on admission. He responded so well to sulfanilamide that he was able to be discharged from the hospital and was able to return to his job as janitor. On this admission he responded well to sulfathiazole but failed to show any change when sulfadiazine was given.

CONCLUSIONS

It appears that certain of the sulfonamides may produce a rather sudden drop in the circulating lymphocytes in lymphatic leucemia. This does not occur in myelogenousleucemia.[10] That this reaction is not of a toxic nature is best indicated by the suddenness of the reponse and by the fact that there is usually an improvement in the patient's condition.

The suggestion of Stamp[4] and Woods[6] that sulfanilamide may interfere with some "growth factor" inhibiting the development of bacteria is worthy of consideration. Is it possible that the drug acting in a similar manner inhibits the multiplication of lymphocytes? If this is so, it is peculiar that similar results do not occur in myelogenous leucemia, and that sulfadiazine failed to produce similar results.

SUMMARY

Four cases of leucemia are presented, all of which showed a drop in the total circulating lymphocytes, and in all but one, clinical improvement, after the administration of sulfanilamide and sulfathiazole.

I wish to acknowledge with thanks the kindness of Dr. R. E. Miller for the follow-up on Case I, and Dr. W. D. Lindsay in allowing Case 3 to be presented.

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THE EFFECTS OF VARIOUS INTENSITIES OF LIGHT ON CERTAIN LABORATORY ANIMALS*

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I. INTRODUCTION

IT IS a common belief that sunlight and well-lighted rooms are healthful. Physicians and lay people assume that sunlight and artificial light are not only beneficial in the treatment and prevention of many diseases but that they promote growth as well. But sunny days and well-illuminated rooms create a desirable environment for most individuals so that the effect of increased light on their well-being is difficult to measure. An obvious lack of control of one's environment makes it yet more difficult to measure the effect of light on the physical, as differentiated from the mental, health of a given group of individuals.

Numerous studies have been made to show the value of ultraviolet therapy, but only a few studies have been made of the effect of visible light on the health and the growth of man and of experimental animals.[6, 24] In our experiment we have attempted to study the influence of various quantities of visible light on the health, the growth, and the behavior of certain laboratory animals.

II. EXPERIMENTAL ROOMS

A. Room Construction.—Three animal rooms were constructed so that no extrinsic light entered them. The rooms measured approximately 16 feet in length, 10 feet in width, and 9 feet in height. Animal cages were placed along one wall of each room, and over them lighting equipment consisting of banks of fluorescent tubes attached to aluminum-covered reflectors was suspended approximately 36 inches above the floors of the cages. The fluorescent tubes were intended to produce approximately 3 foot-candles in one room, 100 foot-candles in the second room, and 1000 foot-candles in the third room. The amount of illumination was measured on the floors of the animal cages. The rooms will be referred to as the 3, the 100, and the 1000 foot-candle rooms, although the levels of illumination did not always reach or remain at the 3, the 100, and the 1000 foot-candle levels (See Chart I).

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B. Lighting.—White fluorescent lamps were used in all three rooms to produce the required illumination. The spectral quality of the light is shown in Chart II. Three 15-watt lamps were sufficient to produce 3 foot-candles, while twelve 30-watt lamps produced more than 90 foot-candles in the second room. Sixty-four of the 40-watt lamps plus sixteen of the 30-watt lamps were needed to produce more than 900 foot-candles in the so-called 1000 foot-candle room. The intensity of illumination was measured periodically with a MacBeth illuminometer. Weekly cleaning of the fluorescent tubes to remove dust films was found to prevent a reduction of light output. Some loss of light intensity was noted, however, as the tubes aged, and tube replacements were necessary.

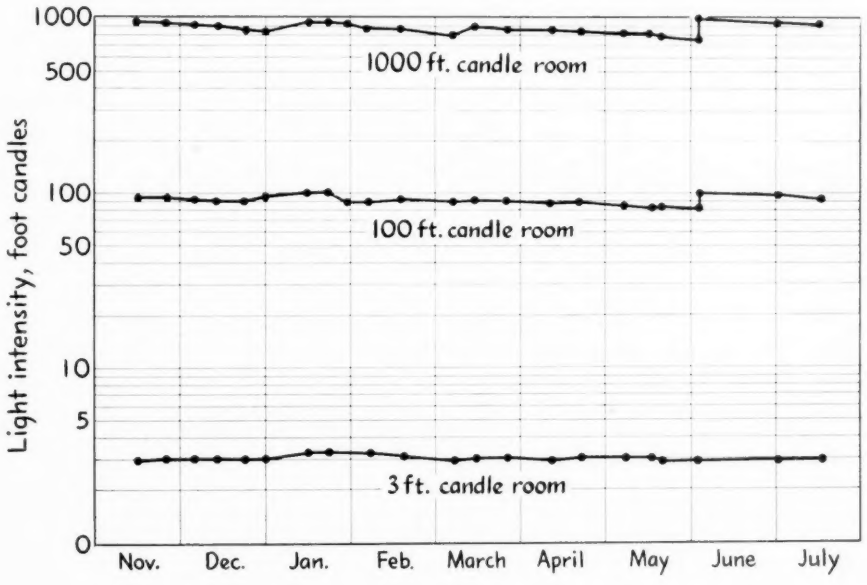

Chart I.—Intensity of light on the floor of the animal cages.

C. Animal Cages.—Animal cages were constructed of thin wire (0.4 mm.) mesh (1 cm. sq.) which permitted a maximum exposure to light; in spite of this precaution, however, there was a loss of approximately 20 per cent as the light passed through the wire mesh. Measurements of light were made inside the animal cages. Glass shields were placed as covers for the cages in the 1000 foot-candle room after removal of the wire tops in an attempt to equalize the radiant energy in the 100- and the 1000 foot-candle rooms. The glass shields were used to absorb and dissipate by convection a considerable portion of the long-wave radiant energy given off by the lamps. The glass covers reduced the radiant energy intensity from 172 British thermal units per square foot per hour to 157 British thermal units per square foot per hour, which figure was considered comparable to 144 British thermal units per square foot per hour found in the 3 and the 100 foot-candle rooms.

D. Temperature and Humidity.—Ventilation of the three rooms was maintained by a 3500 cubic-foot-a-minute fan pulling the warm air of the building into the rooms through light-proof ventilators and exhausting it through a fourth outer room. The air inlets and exhausts were arranged to minimize drafts

on the animals and at the same time to maintain a constant change of air in the rooms. The air velocity in the cages was approximately 15 feet per minute. No attempt was made to control the air temperature or the humidity of the rooms except to avoid extremes. The temperature and the humidity are recorded in Chart III, the peaks seen in the humidity record being due to the washing of the floors and of the fluorescent tubes.

In addition to continuous recordings of the temperature, frequent temperature readings were taken from thermometers placed in the animal cages. In the early days of the experiment the temperature in the 1000 foot-candle room ranged from two to three degrees centigrade higher than in the other rooms, but this elevation subsequently varied less than 1° C. with improved ventilation. These temperature differences were noted after five to six hours of lighting.

E. Time of Exposure.—The animals were exposed to the light in groups varying in size according to the available animal space. The lights were turned on by a time clock at 6 A.M. and turned off at 6 P.M. This period was followed by total darkness. An effort was made to expose the animals from the youngest age at which they could be isolated to young adult life. Although most of the animals were placed in the experimental rooms at 4 to 8 weeks of age, cockerels were placed in the rooms at 48 hours of age. The period of exposure of each animal varied with its health; infection often reduced the duration of the period, for animals were sacrificed if it appeared that infection would be fatal. Healthy animals were sacrificed at the termination of an arbitrarily chosen growth period.

F. Animal Care.—All animals were given superior care and attention. The cages were kept scrupulously clean, and sick animals were promptly placed in isolation cages, where they continued to receive, nevertheless, the same exposure to light. A large Toledo scale was provided for weighing the animals at four-to-five-day intervals.

III. ANIMALS

Guinea pigs, rabbits, rats, and leghorn cockerels were used in the study. These animals were used because of our familiarity with them and because of the ease with which they can be handled. Since some of this group might be considered nocturnal, there is some doubt as to the wisdom of the selection. Many of the guinea pigs and rabbits were albinos, but there were some mixtures of color. The rats were all albinos, and the chickens were all white leghorns.

Animals of both sexes were used, and an attempt was made to obtain litter mates. There was difficulty, however, in obtaining them, and even when they were obtained, the destruction of a litter by loss of one or more animals made the consideration of litter mates of doubtful value in the final analysis. The cockerels were chosen indiscriminately.

The animals received unlimited quantities of food from shielded hoppers which were so constructed as to allow a minimum amount of food to be exposed to the light. The rats were given ground dog chow, while the rabbits and the guinea pigs received rabbit chow. The chickens were given a varied diet consisting of chicken mash, cracked corn, sour milk, oyster shells, and occasionally some ground dog food. Water in unlimited amount was furnished to all animals by means of continuous feed bottles. The water in the cages of the rabbits,

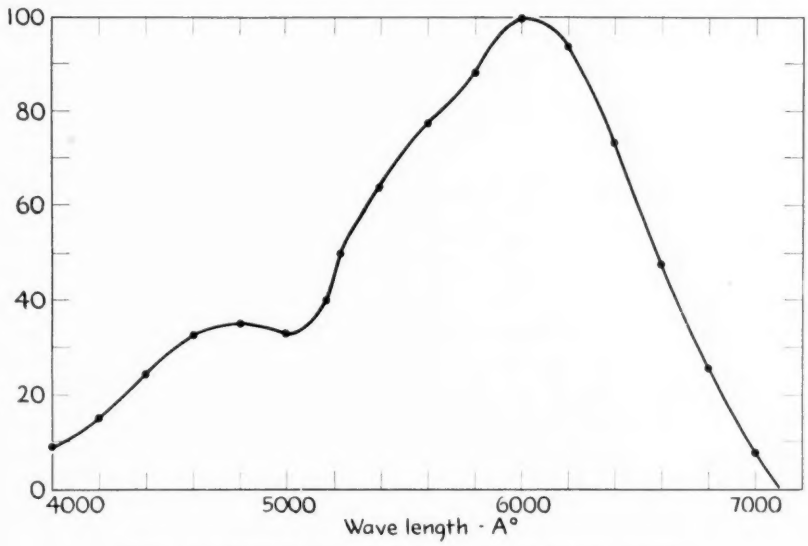

Chart II.—Spectral quality of light in the animal rooms.

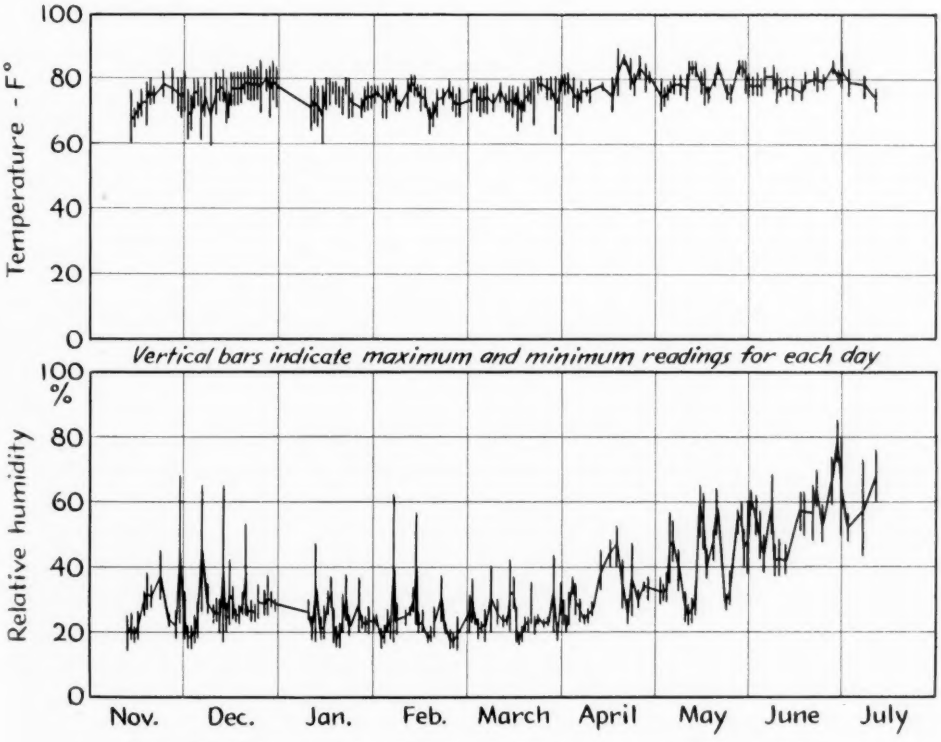

Chart III.—Record of temperature and humidity in the animal rooms.

the guinea pigs, and the chickens was necessarily exposed to light, but the water drunk by the rats was protected in feed bottles. Accurate measurements of the quantity of food and water consumed were not made, but we believe that the amount of food consumed was approximately the same in all rooms. More water was apparently consumed in the 1000 foot-candle room.

IV. RESULTS

A. General Behavior of Animals

1. Activity and Comfort.—The experimental animals were observed closely to determine whether there were any differences in their reactions to exposure to the varying quantities of light. In general there were only slight differences, and these were most evident in comparing the animals in the 1000 foot-candle room with those in the other two rooms. Rats exposed in the 1000 foot-candle room seemed at times to be apathetic, and it was noted that they did not huddle, but tended to lie apart and upon their backs. The rats were most apathetic when the temperature in the 1000 foot-candle room exceeded that of the other rooms, so we attributed some of the differences in activity to the differences in temperature. Rabbits and guinea pigs seemed to be equally active in all rooms. The cockerels in the 1000 foot-candle room had a tendency to pant more readily than the birds in the 3 and the 100 foot-candle rooms. All experimental animals in the 100 foot-candle room were as comfortable and active as animals in the 3 foot-candle room. Apparently the intensity of light does not prove uncomfortable to animals if the temperature is not elevated.

2. Health.—The health of the animals was as good as could be expected in view of the fact that animal quarters were limited. In all rooms some of the rabbits developed numerous attacks of severe diarrhea associated with anorexia and marked loss of weight. These infections proved to be highly contagious so that prompt isolation was necessary. Though a few of the animals succumbed to infection, the majority of them recovered without specific therapy. The considerable weight loss by the rabbits during infection was promptly regained with improved health.

The rats remained in excellent health throughout the experiment, and losses were negligible. Respiratory infections and snuffles were at a minimum while the growth and the gain in weight were constant.

The guinea pigs remained in good health in all experimental rooms during the first ten to fifteen days of the experiment, but after this period they developed shaggy hair, anorexia, diarrhea, muscle weakness, and marked loss of weight, and were sacrificed. We believe that the picture was similar to experimentally produced vitamin C deficiency.

The cockerels were in excellent health throughout the entire experiment. A few of the unfeathered birds succumbed in the early days of the experiment because of low temperature for chicks. Subsequently the chickens were vigorous and very active. Those in the 1000 foot-candle room panted with an elevation of temperature.

3. Sensitivity to Light.—The experiment provided an opportunity for observing the reactions of the animals to high levels of illumination. Our daily observations should be of some practical value, for animals may be more sensitive to light than man. We paid particular attention to behavior, to at-

tempts to hide, and to the appearance of the feathers or of the animals' skin. As far as we could determine, all animals appeared to be unaffected by the high levels of illumination in the 100 and the 1000 foot-candle rooms. No attempts to hide from the light were noted. Examination of the skin of the animals revealed the color, the texture, and the vessels to be comparable in all rooms, and no evidence of abnormal pigmentation was present. The feathers of the chickens were normal to gross examination; the combs of the chickens in all rooms appeared to be paler and larger than normal. This latter change was attributed to the minimal amount of ultraviolet rays present in the light.

	MALE					FEMALE				
	Animal number	Color	Total weight gain (grams)	Growing days	Average daily weight gain (grams)	Animal number	Color	Total weight gain (grams)	Growing days	Average daily weight gain (grams)
3 ft. candle room	1	B	1203	86	13.9	4		187	8	23.3
	2	B	1070	56	19.1	5		993	41	24.2
	3	B	1361	56	24.3	6		1078	41	26.3
						7		594	29	20.4
						8		547	25	21.8
						9	W	3007	86	34.9
						10	W	436	55	7.9
						11	W	218	10	21.8
						12	W	880	38	23.1
	Average		*1211.3*	*66.0*	*19.1*	*Average*		*882.2*	*37.0*	*22.63*
100 ft. candle room	13		325	9	36.1	14		649	29	22.4
						15		414	14	29.5
						16		1078	40	26.9
						17		498	41	12.1
						18		461	25	18.5
						19	WB	675	47	14.3
						20	WB	2027	87	23.3
						21	W	1616	39	41.4
						22	W	1686	39	43.2
	Average		*325.0*	*9*	*36.1*	*Average*		*1011.5*	*40.1*	*25.62*
1000 ft. candle room	23	W	2089	89	23.4	25		584	41	14.24
	24	B	1000	58	17.2	26		222	10	22.2
						27		626	25	25.0
						28		623	25	24.9
						29	B	2152	88	24.4
						30	W	518	12	43.1
						31	W	1421	40	35.5
	Average		*1544.0*	*73.5*	*20.30*	*Average*		*892.2*	*34.4*	*27.04*

Chart IV.—Record of the growth of female rabbits.

4. Growth.—Charts IV through X furnish evidence of the growth of the experimental animals. The rate of growth per day was considered a fair index of the effect of the varying quantities of light upon the animals, and comparison of the growth of all animals in the 3, the 100, and the 1000 foot-candle rooms has been made. Only the animals that survived the experimental period and those living sufficiently long to be significant are compared in the charts.

a. Rabbits.—Examination of Chart IV reveals the growth record of 25 female rabbits, an obviously small number. The growth period of the animals was variable because of the loss of some of the animals by infection. The

average daily weight gain was practically the same; the average gain of the animals in the 1000 foot-candle room, 27.04 grams, was only slightly greater than that of the animals in the 100 and the 3 foot-candle rooms, 25.62 and 22.63 grams respectively. The comparison of litter mates, as suggested before, did not prove to be useful. Only a few male rabbits survived the experimental period so that any study of their relative growth would be of little value. In the course of the experiment it became evident that the maintenance of health in rabbits in small quarters is difficult.

	MALE					FEMALE				
	Animal number	Color	Total weight gain (grams)	Growing days	Average daily weight gain (grams)	Animal number	Color	Total weight gain (grams)	Growing days	Average daily weight gain (grams)
3 ft. candle room	1	W	31	10	3.0	9	W	59	16	3.7
	2	W	62	16	2.5	10	W	44	14	3.1
	3	W	92	24	3.8	11	W	61	17	3.6
	4	W	71	16	4.4	12	W	57	16	3.5
	5	W	35	17	2.0	13	W	74	17	4.3
	6	W	48	17	2.8	14	W	13	15	0.86
	7	W	84	17	4.9	15	B	16	15	1.06
	8	R	20	16	1.25					
	Average		*55.3*	*16.6*	*3.08*	*Average*		*46.2*	*15.7*	*2.86*
100 ft. candle room	16	W	77	24	4.2	20	W	72	14	4.0
	17	W	51	21	2.4	21	W	64	17	3.7
	18	W	64	17	3.7	22	W	48	15	3.20
	19	R	49	15	3.26	23	W	10	7	1.42
						24	B	23	15	1.53
	Average		*60.2*	*19.2*	*3.39*	*Average*		*43.4*	*13.6*	*2.77*
1000 ft. candle room	25	W	45	17	2.6	29	W	23	14	1.6
	26	W	36	14	2.5	30	W	94	24	3.8
	27	W	89	17	5.2	31	W	15	12	1.2
	28	R	43	15	2.86	32	W	96	29	3.3
						33	W	43	17	2.5
						34	W	40	16	2.5
						35	W	52	14	3.7
						36	W	15	15	1.0
						37	B	32	15	2.13
	Average		*53.2*	*15.7*	*3.15*	*Average*		*45.5*	*17.3*	*2.41*

Chart V.—Record of the growth of guinea pigs.

b. Guinea Pigs.—The 36 guinea pigs placed in the experimental rooms apparently developed scurvy so that for the purpose of this experiment the group was unsatisfactory. The healthy guinea pigs placed in the experimental rooms were given dog chow and were not allowed lettuce or similar food because of the desire to avoid an exposure of the food to light. As previously stated, the animals remained healthy for a period of ten to fifteen days, after which time they developed shaggy hair, some muscular weakness, and subsequently a marked fragility of the bones. Litter mates in all three rooms became sick at approximately the same time. Since the pigs received no other food than the dog chow, we were of the opinion that they developed vitamin C deficiency. Analysis of Chart V shows that the guinea pigs were in the experimental rooms approxi-

mately two weeks. The brief exposure period and the vitamin C deficiency present make comparison of these animals unsatisfactory. The average daily weight gain during their growth period was 3.08 grams for the male pigs in the 3 foot-candle room and 3.39 and 3.15 grams respectively for the male pigs in the 100 and the 1000 foot-candle rooms. Similar small differences were noted in the female pigs, but the gain was greatest in the 3 foot-candle room and smallest in the 1000 foot-candle room. A comparison of the litter mates showed wide variations in the average weight gain (Chart V).

	MALE				FEMALE			
	Animal number	Total weight gain (grams)	Growing days	Average daily weight gain (grams)	Animal number	Total weight gain (grams)	Growing days	Average daily weight gain (grams)
3 ft. candle room	1	85	29	2.93	17	34	33	1.03
	2	62	33	1.87	18	154	81	1.90
	3	77	33	2.33	19	84	51	1.64
	4	138	43	3.20	20	93	51	1.82
	5	169	43	3.93				
	6	137	43	3.18				
	7	111	43	2.71				
	8	121	43	2.81				
	9	48	33	1.42				
	10	145	43	3.39				
	11	66	33	2.00				
	12	159	80	1.98				
	13	192	80	2.40				
	14	175	80	2.18				
	15	118	50	2.36				
	16	159	50	3.18				
	Average		47.3	2.61	Average		54	1.59
100 ft. candle room	21	69	33	2.09	34	35	33	1.06
	22	62	33	1.87	35	39	33	1.18
	23	65	33	1.96	36	34	32	1.03
	24	85	43	1.97	37	130	81	1.72
	25	76	43	1.76	38	154	81	1.90
	26	62	43	1.44	39	116	82	1.41
	27	79	33	2.39	40	86	52	1.65
	28	139	43	3.73	41	80	52	1.53
	29	47	33	1.31				
	30	309	80	3.86				
	31	170	81	2.09				
	32	161	51	3.15				
	33	137	51	2.68				
	Average		46.1	2.33	Average		55.7	1.43
1000 ft. candle room	42	49	33	1.48	57	28	33	0.84
	43	56	33	1.69	58	29	33	0.87
	44	77	43	1.79	59	25	24	1.04
	45	128	43	2.97				
	46	76	43	1.76				
	47	91	43	2.11				
	48	116	43	2.69				
	49	58	33	1.75				
	50	112	43	2.60				
	51	62	33	1.87				
	52	145	83	1.75				
	53	178	83	2.14				
	54	171	83	2.06				
	55	153	53	2.88				
	56	127	53	2.40				
	Average		49.6	2.12	Average		30	0.91

Chart VI.—Record of the growth of albino rats.

c. Rats.—The 56 rats proved to be satisfactory animals in this experiment. These animals remained in good health throughout the experimental period, and most of them were sacrificed after being in the experimental rooms approxi-

mately fifty days. Chart VI gives a comparison of the growth of the 41 male rats. It reveals that the average daily weight gain of the male rats in the 3 foot-candle room (2.61 grams per day) exceeded that of the male rats in the 100 foot-candle room (2.33 grams) and that of the male rats in the 1000 foot-candle room (2.12 grams). A similar trend is noted in the small group of female rats. The average daily weight gain in the 100 and the 1000 foot-candle rooms was less than that in the 3 foot-candle room. A comparison of litter mates likewise reveals a tendency for litter mates in the 3 foot-candle room to gain more per day than litter mates in the 100 and the 1000 foot-candle rooms. The average daily weight gain of each rat is shown graphically in Chart VII. From a study of this chart it is evident that few animals in the 1000 foot-candle room gained as much as the animals in the 100 and the 3 foot-candle rooms. A study of the growth curves in Chart VIII shows the growth of animals was relatively uniform throughout the experimental period. From this chart one would assume that the beneficial or harmful effect of varying quantities of light was not evident in any particular period of the experiment.

d. Chickens.—A few chicks died in the early days of the experiment, probably because of relatively low temperatures for unfeathered birds, and the hens were discarded from the experiment when they were identified. The remaining 56 cockerels were exposed for approximately eighty-three days and then sacrificed. The health of all birds was good throughout the experiment.

A comparison of the growth of the chickens is tabulated in Chart IX. The cockerels in the 3 foot-candle room gained less (7.89 grams) than those in the 100 foot-candle room (8.07 grams) and more than those in the 1000 foot-candle room (7.47 grams). Chart IX indicates that the different levels of illumination in the experimental rooms did not produce significant variation in the rate of growth of cockerels. Chart X reveals the growth curves of the cockerels to be similar.

B. Specific Observations of Animals

1. Eyes.—The eyes of the animals were frequently observed to study the effect of various quantities of light on the conjunctivae, the wink reflex, and the fundi. Examination of the eyes was done carefully because any possible damage due to light could be more readily detected in the eye than elsewhere.

The conjunctivae of the animals in all rooms were normal to gross examination. There was no tendency of any of the animals to blink frequently or to close their eyes in an attempt to avoid the light.

Fundoscopic examinations were done on a number of rabbits, and several were found to have increased pigmentation resembling chorioretinitis. These animals, however, were not examined until after they had been exposed to the light, and the pigment may have been present prior to their exposure to light in the experimental rooms. In two rabbits a clouding developed in the media, making a clear view of the fundi impossible. Slit-lamp examination of one localized the difficulty in the lens; the other animal died of infectious diarrhea before examination. The fundi of two litters of rabbits, six animals, were studied before their exposure to light in the experimental rooms. Although all six rabbits were albinos, a few pigmented hairs were found on the tips of their tails and on their ears, and some of the group were found to have partially

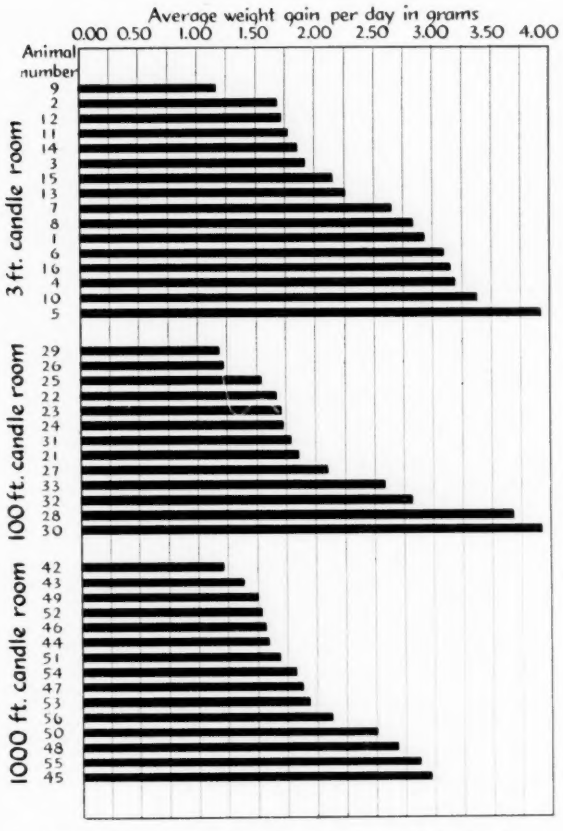

Chart VII.—Average daily gain in weight of rats.

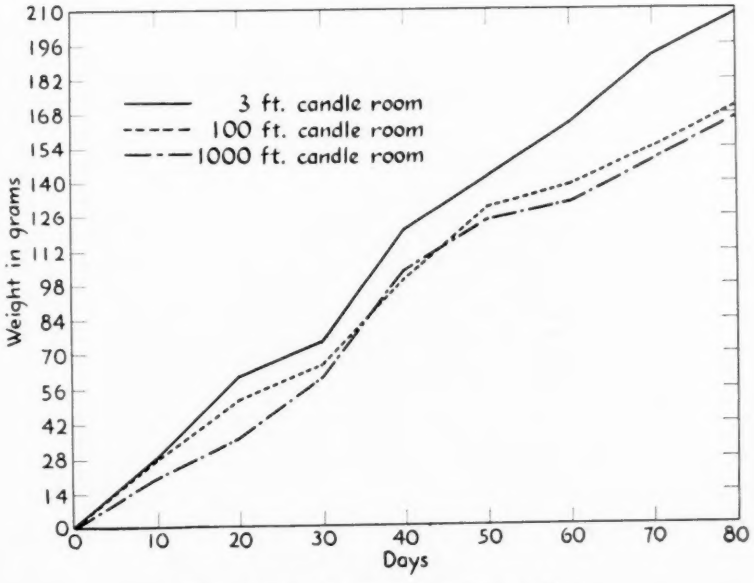

Chart VIII.—The growth curve of rats.

pigmented fundi. The amount of pigment in the fundi did not increase in any of these rabbits after their exposure to light in the 3, the 100, or the 1000 foot-candle room. It was noted that normal black rabbits have a large amount of pigment in their fundi; hence the presence of pigment after exposure to light need not be considered abnormal.

The examining ophthalmologist, Dr. Frances MacCraken, could find no evidence that the eyes of the rabbits were injured by high levels of illumination, but further study on this subject should be done. The eyes were removed for histologic examination.

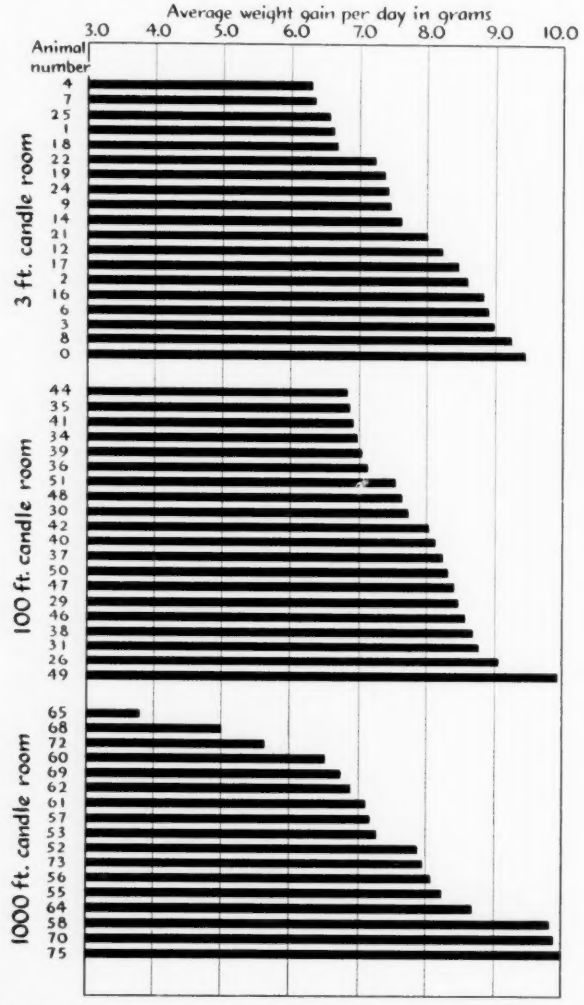

Chart IX.—The average daily gain in weight of cockerels.

2. Hair and Feathers.—The hair and the feathers of the experimental animals in the various rooms were compared. No differences could be detected in the texture, the length, the thickness, and the color of the hair or the feathers. The hair of the shorn rabbits and guinea pigs, however, was found to grow most rapidly in the 1000 foot-candle room and least rapidly in the 3 foot-candle

room (Figs. 1, 2). Many rabbits were repeatedly shorn to check this observation. The variation was constant in litter mates. Numerous biopsies of the shorn areas were taken for histologic study.

3. Laboratory Procedures.—Only a few laboratory studies were made. Hemoglobin determinations and blood smears were made from a few rats, a few rabbits, and some chickens. Both hemoglobin levels and blood smears were found to be normal and comparable in animals from all the experimental rooms.

C. Autopsy Studies.—Autopsies were done on all animals. The majority were sacrificed at the end of the experimental period, but a few died in the course of the experiment.

1. Gross Studies.—Rabbits and rats showed no significant gross variations; occasionally a few small areas of bronchopneumonia were noted. Examination of guinea pigs revealed extensive hemorrhages into the muscles of the hind legs and extreme fragility of the bones. The remainder of the organs were essentially normal to gross inspection. The chickens were found to be normal on gross examination.

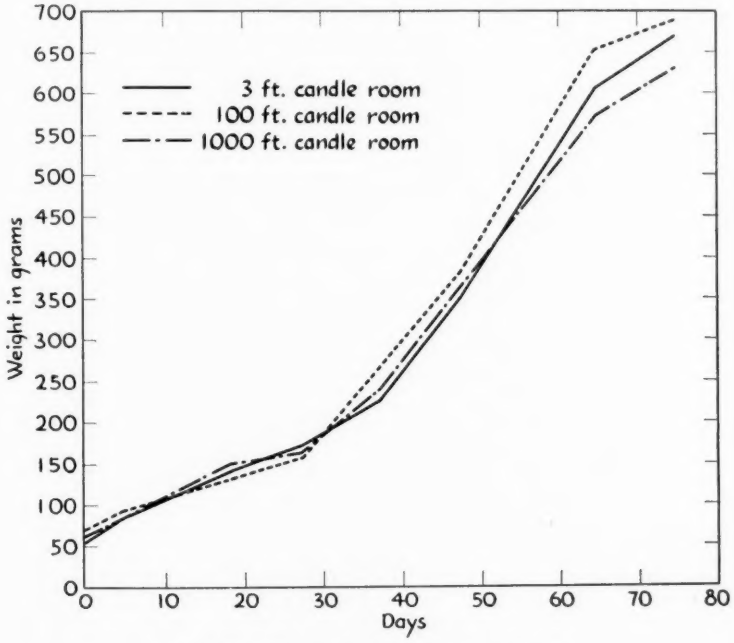

Chart X.—The growth curve of cockerels.

2. Microscopic Studies.—Sections from all organs were placed in 10 per cent formalin, sectioned, and stained with hematoxylin and eosin. Pieces of bone (the femurs were usually selected) were decalcified for microscopic study, and skin sections and skin biopsies were prepared from all animals. Examination of the prepared slides did not reveal any significant changes in the organs that one would not find in the study of control animals. Examination of the bones and the muscles of guinea pigs revealed findings similar to those previously described in experimental scurvy.[33] Skin biopsies taken from rabbits

in the experimental rooms revealed a few lymphocytes around some of the vessels of the corium. The number and the distribution of the lymphocytes did not vary according to the exposure of the animals to different intensities of light.

V. DISCUSSION

A. Health.—Physicians frequently advise increased exposure to artificial light and sunlight, but the evidence supporting the beneficial effects of light has been questioned (Clausen).[6] Although many clinical and some laboratory experiments call attention to the value of light, by which are most often meant rays of less than 3300 angstroms, most clinical and laboratory studies have shown that man and animals are able to continue in good health under restricted amounts of light if they are provided adequate diets.

Pearce and Van Allen[30] found evidence that the health of rabbits was not impaired by darkness, but that the rabbits exposed to constant light gained more weight than the unexposed group. From post-mortem observations they found no evidence that spontaneous lesions occurring in the experimental animals were related to the amount of light to which they had been exposed. Mackay[27] could find little evidence that infants exposed to mercury vapor quartz lamps grew faster or were in better health than a control group of children.

The anemic appearance of those living and working in poorly lighted homes and factories does not always indicate poor health. Little anemia has been found in animals working in mines.[18]. Rats placed in dark rooms were found to have a decreased number of blood platelets, but their general health remained good.[8] Laboratory studies on animals subjected to long periods of darkness have shown very little variation in the hemoglobin percentage or in the red cell count if the diet was adequate. Dogs placed in darkness for brief periods have shown an initial decrease in red blood corpuscle counts but a subsequent quick return to normal. Clinicians, on the other hand, have observed a rapid increase in hemoglobin and in red blood cells in anemic individuals exposed to solar and quartz mercury lamp radiations. Miles and Laurens[28] have shown that irradiations of anemic animals with ultraviolet rays are followed by an increase in the cellular elements and in hemoglobin; in this study, however, the changes were probably minimal and insignificant.

The general belief that increased quantities of light perhaps produce better health has led to the use of artificial light in the treatment of wounds and in the prevention of infections. Sunlight has been found to retard the growth of bacteria,[11] and ultraviolet radiation is advocated to reduce the frequency of infections in hospital operating rooms.[19, 23] Hill and Clark,[22] however, were unable to show that ultraviolet rays are beneficial to rats infected with pneumococci. In recent studies Buchbinder, Solowey, and Phelps[5] report the effect of various types and quantities of light on the growth of several strains of streptococci. They found bright sunlight to be a more powerful lethal agent on growing bacteria than daylight or artificial light. Their experiment indicates that the lethal action of light on bacteria is somewhat proportionate to the quantity of light and is related to the spectral quality of the light. The authors suggest that the suppression of the growth of bacteria by light may play an important role in the control of upper respiratory tract infections.

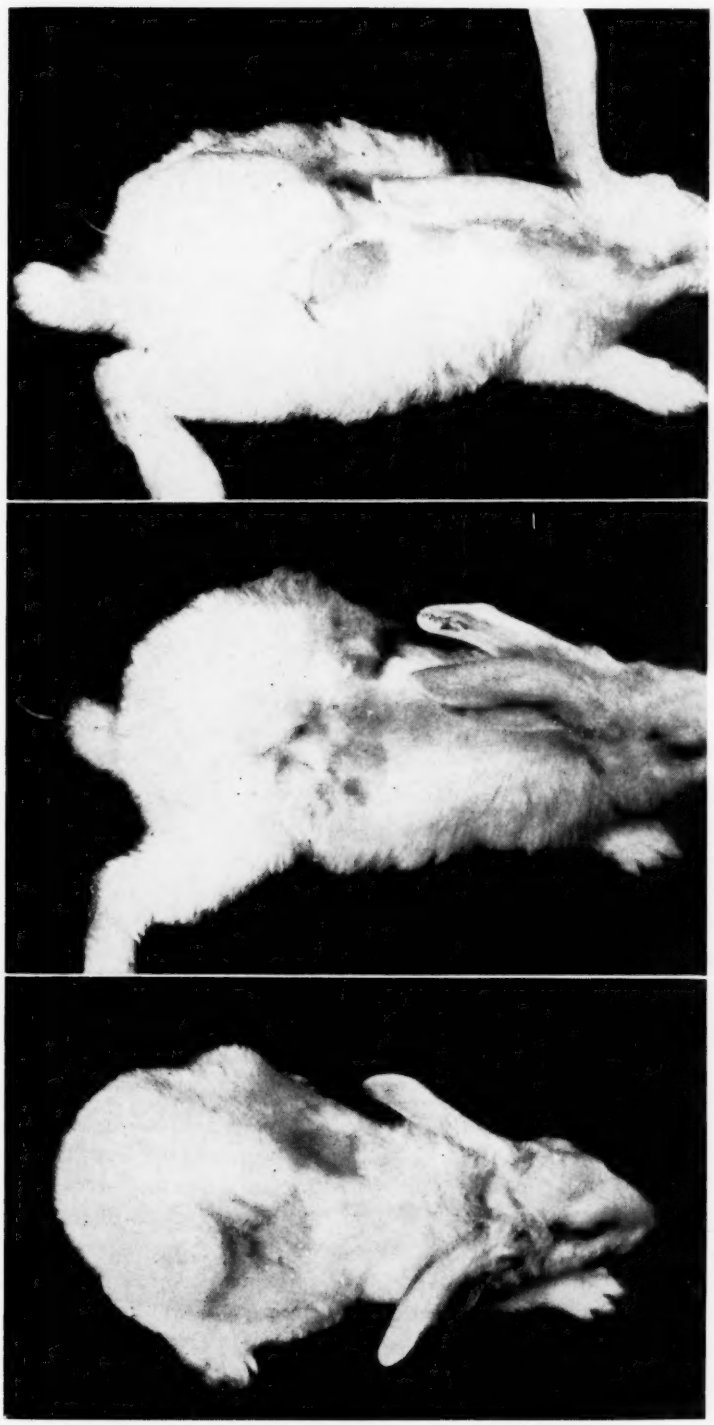

Fig. 1.—Litter mates from the 3, the 100, and the 1000 foot-candle rooms. Photograph taken five days following shaving.

Observers disagree concerning the effect of light on the healing of experimental wounds. Coburn and Cowles[7] found that experimental wounds of rats heal as well in darkness as in sunlight. On the other hand, Leo and Vaucher[26] and other experimenters as well have shown that wounds exposed to sun heal better than those covered. Crile[9] found artificial light from Mazda lamps a helpful adjunct in the treatment of wounds, but the beneficial effects in this instance could be due to the increased heat. Clausen,[6] after carefully considering controlled clinical studies and laboratory experiments, expressed doubt that light favors growth and promotes resistance to infections.

B. Growth.—Most investigators have found evidence that sunlight and artificial light stimulate the rate of growth of man and of experimental animals. This observation has proved of practical value in that chicks are often exposed to artificial light during their growth period.[20] In reviewing the reported experiments on the comparative rate of growth, one finds that investigators have usually compared the growth of animals in darkness or in minimal light devoid of ultraviolet rays to the growth of animals exposed to sunlight or to artificially produced ultraviolet rays.

Wade Brown[4] placed groups of normal rabbits in an experimental room in which neon tubes were added to produce 20 to 50 foot-candles of light. The rabbits in this room gained more weight than other rabbits grown in a dark room and more than rabbits grown in sunlight. In this experiment two groups of animals were exposed to ultraviolet rays while the quantity of light in the group exposed to sunlight is unknown. In comparable studies albino rats grown in darkness were found to grow more slowly than rats grown in sunlight,[25] and rabbits exposed to 425 foot-candle illumination were found to gain more than rabbits grown in dark rooms and in control rooms.[31] Bovie[3] noted that chickens that were raised in sunlight passed through a glass roof (probably devoid of ultraviolet rays) grew poorly compared to chickens raised in uninterrupted sunlight, and Borissow[2] found that dogs and rabbits grew better in adequate than in dim light.

Degkwitz[14] found that puppies raised in darkness appeared to be as healthy as control animals, but they showed a measured impoverishment in total ash, calcium, phosphorus, and magnesium. In a study of the growth of rats on deficient diets, Goldblatt and Soames[17] found that the rats irradiated with quartz mercury lamps grew more than the nonirradiated animals.

Northrop[29] calls attention to the fact that most reported experiments fail to show the effect of various quantities of light on the rate of growth of experimental animals. He exposed imagoes to varying intensities of light and discovered that there was no change in the rate of growth in intensities up to 600 meter candles, but he found that above that level the length of life of the imagoes decreased rapidly. After exposing flies to 10,000 meter candles, Northrop found their life span to be only 3.9 days compared to an average of 13.5 days for flies kept in the dark. He was unable to find evidence, however, that the absence of light in 230 generations had any effect on the duration of life or on the ability of the flies to resist infections. Higgins and Sheard[21] noted that frogs exposed to quartz mercury lamps were stimulated in the first twenty-four hours but that subsequently they showed a retardation of growth.

In a study of 100 healthy children Frank[16] was unable to correlate the growth periods with temperature, precipitation, or the amount of sunlight to which the children were exposed. Clinical studies are unsatisfactory because of the many factors beyond the control of the observer.

In our study we could find no evidence that an increased amount of light devoid of ultraviolet rays promotes growth. High levels of illumination, however, apparently produce no unfavorable influence on the health of experimental animals.

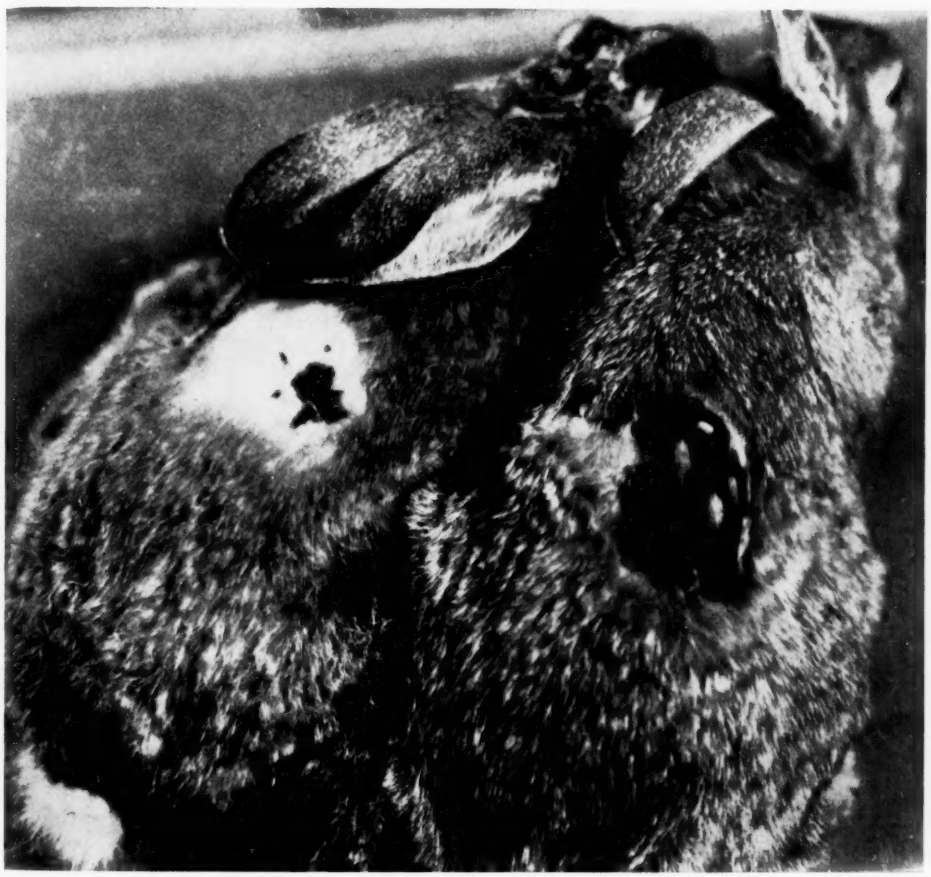

Fig. 2.—Litter mates from the 100 and the 1000 foot-candle rooms four days after shaving.

C. Eyes.—Artificial light rarely produces clinical symptoms or pathologic effects, but light improperly directed may be uncomfortable to the eye and in a few instances produce severe damage. Overstimulation produced by a constant bright light is thought to result generally in only physiologic fatigue and only very seldom in any detectable tissue damage.[13] The action of light upon the eye has been shown to be dependent on its wave length and on its intensity.[12] The shorter radiations, 3150 angstroms or less, are largely absorbed by the cornea or the lens while the radiations of the visible spectrum are more likely to be transmitted to the retina. Conjunctival and corneal lesions have been noted when the eye was exposed to strong radiations from artificial illuminants,

as mercury quartz lamps, or when the eye was exposed to reflected sunlight. Very long wave lengths may produce damage to all layers of the eye, the damage varying in degree with the intensity of the incident illumination and with the boundaries of transmission and absorption found to be different in different individuals. It is believed that the human eye is more transparent in the visible range than that of most nocturnal animals.

D. Sexual Maturity.—It is a common belief that natives in the tropics mature earlier than individuals living in temperate zones. In a careful clinical study Sundstroem[32] found that Anglo-Saxon girls in the tropics reached maturity at approximately the same age as girls in England; hence he believes that early maturity is a racial characteristic. Experimental studies, however, indicate that exposure to increased quantities of light produces early maturity and an increased size of the endocrine glands.

Fiske[15] raised groups of immature female rats in darkness, ordinary light, and continuous light. She noted that the immature female rats kept in light from birth or from the twenty-first day of life came into sexual maturity somewhat earlier than rats grown in ordinary laboratory light and much earlier than rats grown in complete darkness. In her group of males Fiske found at autopsy that the pituitaries, the testes, and the seminal vesicles were largest in the rats exposed to the maximum quantity of light. The difference in size was seen to be be greatest at 150 days; after 150 days the weights of the organs became comparable to the weights of organs from rats raised in dark rooms. Fiske points out that her study indicates a stimulating effect by light on the pituitaries of both male and female rats. Lack of light, in her opinion, favors a decrease in follicle-stimulating hormone and an increase in luteal hormone in the female. Similar conclusions were reached by Dawson[10] in a study of cats and by Benoit;[1] in the latter's study the testicles of immature ducks showed a rapid increase in size proportionate to the amount of illumination to which they were exposed.

In our study no careful observations were made to determine the relative time of development of maturity of animals exposed to different quantities of light. At post-mortem careful studies of the organs of the endocrine system were not made, but no gross deviations were seen in the various rooms.

E. Hair Growth.—An increased rate and amount of hair growth has been noted on the arms of attendants working in solariums. Brown[4] found that shorn albino rabbits exposed to neon lights showed an active proliferation of hair follicles, while rabbits living in the dark showed delayed, incomplete, or irregular growth. He attributed the effect to ultraviolet radiations.

Sundstroem,[32] after carefully measuring and weighing his clipped hair and nails, found that they grow 10 per cent more slowly in the tropics. He indicated that a slower rate of growth would be expected in all furry animals in warm climates.

We were able to confirm the findings of Brown[4] in that our shaved animals showed a more rapid hair growth when exposed to increased quantities of light. We did not, however, find a proliferation of hair follicles in the sections of skin taken by biopsy or obtained at autopsy. We believe the stimulus for increased hair growth may be central rather than peripheral.

F. Post mortems.—Careful post-mortem studies were reported by Pearce and Van Allen[31] of animals exposed to mercury quartz vapor lamps. The

organs of rabbits in the light rooms weighed less than those of rabbits in the control rooms. Small lesions noted in the organs were considered insignificant. Other studies of animals after exposure to light showed no differences in the organs of animals exposed to various quantities of light.

At the autopsies of the animals in our experiment gross inspection showed no differences in the organs of animals from the 3, the 100, and the 1000 foot-candle rooms. In a few animals small lesions were found in some organs, but they were irregular in distribution and did not appear to have any relation to the amount of light to which the animals had been exposed. Examination of slides prepared from the different organs showed them to be no different from the organs of control animals. One could conclude that varying quantities of light produce no obvious tissue damage.

VI. SUMMARY

Groups of rats, rabbits, guinea pigs, and chickens were exposed to different intensities of light in three experimental rooms. The animals received unlimited quantities of similar food in each room, and all were exposed to light an equal length of time daily. All animals were observed frequently to note any difference in behavior due to exposure to various amounts of light, and each animal was weighed frequently.

Animals were sacrificed at the end of an arbitrarily chosen experimental period if they had not died of an intercurrent illness. Autopsies were performed upon all animals, and tissues were obtained for microscopic study.

The health of the animals in the 3, the 100, and the 1000 foot-candle rooms was approximately the same. No differences in behavior of the animals could be detected, and the gain in weight of the various animals in the different experimental rooms was approximately the same. There was a noticeably increased rate of hair growth in animals exposed to higher intensities of light. Autopsy examinations revealed no unusual differences in the organs of animals exposed to various quantities of light.

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UREASE ACTIVITY OF PROTEUS AND SALMONELLA ORGANISMS*

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ONE of the most important details of routine enteric bacteriology is the separation of nonpathogens from pathogens in the process of cultural elimination. It is usual for bacteriologists to transfer isolated colonies from differential plate media to triple or double sugar slants and judge from the reaction of the slants whether or not the organisms should be studied further. In the elimination process the greatest trouble to the worker results from those organisms which produce an alkaline surface on the slants, an acid butt and gas.

There are several possibilities as to the identity of such an organism. It may belong to the paracolon group, consisting of slow lactose- and sucrose-fermenting organisms with general coliform characteristics. It may belong to the proteus group whose members are nonlactose-fermenting, display a variable ability to ferment sucrose, and whose swarming tendencies are inhibited on differential media. It may belong to the genus Salmonella.

Among those working with animal fecal pathogens, the problem of separation of Proteus bacteria from Salmonella-like organisms is particularly burdensome because of the many Proteus strains encountered.

A test for swarming will prove of considerable value in discovering Proteus strains but, unfortunately, such a test is not entirely reliable. A small percentage of Proteus organisms have no ability to swarm; some Proteus strains are not highly motile and give an inconclusive reaction on two per cent agar. It has been our experience that some Salmonella strains on moist agar simulate the swarming activity of Proteus.

It is known that the urease activity of Proteus organisms and the nonurease activity of Salmonella provide a method of separating the commonly encountered members of the genus Proteus from organisms of the Salmonella group.

Numerous investigators have attested to the urea-decomposing ability of Proteus organisms. Of particular interest are the observations of Moltke[1] and of Rustigian and Stuart[2] who have made extensive studies on urea decomposition. Moltke examined 194 strains of maltose-fermenting and nonmaltose-fermenting strains of Proteus and found that all attacked urea. In 1941 Rustigian and Stuart[2] reported that all members of the genus except *Proteus hydrophilus*, *Proteus ichthyosmius*, and *Proteus bombycis* were capable of decomposing urea with formation of ammonia and subsequent production of alkali. They agreed with St. John-Brooks and Rhodes[3] that *Proteus hydrophilus* and *Proteus ichthyosmius* should be excluded from the genus. Rustigian and Stuart would also exclude *Proteus bombycis* from the genus for its inability to decompose urea.

The paracolon group of organisms has apparently not been studied for urease activity except by Ashworth[4] who tested 30 paracolon strains and found them unable to attack urea.

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According to Bergey,[5] organisms of the Tribe Salmonelleae do not hydrolyze urea.

In a study on Salmonelliasis in infants, Hormaeche and Peluffo[6] described a simple medium consisting of Simmon's agar plus urea which they employed for differentiating Proteus and Salmonella organisms. They stated that the latter exert no action on the urea.

In our search of the literature we have been unable to find a comprehensive list of Salmonella strains which have been examined for urease activity. It is the purpose of this paper to supply such a list in the hope that other workers will be encouraged to use a test for decomposition of urea as an aid in enteric bacteriology.

MEDIA

Three media were used in testing the organisms listed in this paper.

Formula 1 was the synthetic medium of Rustigian and Stuart,[2] containing 2 per cent urea (Baker's, C.P.),* 0.01 per cent yeast extract (Difco), and M/15 primary and secondary phosphate buffers (Sorensen) in distilled water to give a final pH of 6.8. Sterilization was obtained by filtration through Seitz pads.

Formula 2 was devised in our laboratory. Its ingredients were as follows: 2 per cent urea (Baker's, C.P.), 0.1 per cent KH_2PO_4, 0.1 per cent K_2HPO_4, 0.5 per cent NaCl and 1.0 per cent ethyl alcohol (95 per cent). The pH was adjusted to 7.0. Sterilization was accomplished by filtration through a Seitz pad.

Both media were dispensed in 3 to 5 c.c. amounts in sterile 5 × 5/8 inch culture tubes.

Formula 3 was the solid medium of Ashworth[4] dispensed in the form of slopes. Its ingredients were: agar 2.5 per cent, distilled water, K_2HPO_4 0.1 per cent, $CaCl_2$ 0.01 per cent, $Mg\ So_4 \cdot 7H_2O$ 0.01 per cent, NaCl 0.01 per cent, $FeCl_3$ 0.001 per cent, beef extract (Difco)† 0.5 per cent, brom thymol blue (1.5 per cent) 1 per cent, and urea 2.0 per cent. All ingredients, except urea, were dissolved in distilled water, and the base was sterilized by a temperature of 121° C. for twenty minutes. Sufficient 20 per cent urea solution was added to make a final concentration of 2 per cent urea.

METHOD OF TESTING

Organisms under test for urease activity were inoculated by loop from a 24-hour growth on veal infusion agar slants to the liquid media of Formulas 1 and 2 and to the slanted surface of Formula 3. After incubation for 24 hours at 37° C. the liquid media were tested for formation of $(NH_4)_2CO_3$ by the addition of several drops of brom thymol blue indicator. An intense blue color was accepted as evidence of the breakdown of urea with formation of alkali. The solid slants of Ashworth's medium (Formula 3) were observed for change in reaction 24 hours after inoculation of test organisms.

TEST ORGANISMS

Thirty strains of Proteus from animal and human sources were tested for urease activity. Their reactions in urea media are given in Table I. Seven of

*Rustigian and Stuart specified Merck's urea.
†Ashworth specified Lemco beef extract.

the cultures came originally from the American Type Culture Collection; however, of these, *Proteus hydrophilus* I and II did not possess the characteristics of this species. *P. hydrophilus* I gave the biochemical reactions of *Proteus vulgaris*, with the exception of rapid gelatin liquification; *P. hydrophilus* II resembled *Proteus mirabilis* in its reaction. Both organisms produced swarming on 2 per cent agar. The Proteus strains from animal sources were obtained from the collection of Mr. C. W. Darby of Michigan State College.

TABLE I

REACTION OF PROTEUS ORGANISMS IN THREE UREA MEDIA

CULTURES	SOURCE	FORMULA 1 (RUSTIGIAN AND STUART)	FORMULA 2	FORMULA 3 (ASHWORTH)
S 779 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
S1002 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
P. hydrophilus I*		Alkaline	Alkaline	Alkaline throughout
P. hydrophilus II*		Alkaline	Alkaline	Alkaline throughout
P. ichthyosmius*		Neutral	Neutral	Alkaline surface
S1008 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S 771 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
S 911 P. mirabilis	Turkey	Alkaline	Alkaline	Alkaline throughout
S1149 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S1010 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S 373 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
S 983 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
S1021 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S1161 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
P. mirabilis I*		Alkaline	Alkaline	Alkaline throughout
P. mirabilis II*		Alkaline	Alkaline	Alkaline throughout
P. ammoniae*		Alkaline	Alkaline	Alkaline throughout
S 599 P. mirabilis	Pig	Alkaline	Alkaline	Alkaline throughout
S1003 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
P. americanus*		Alkaline	Alkaline	Alkaline throughout
S 429 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
S1144 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S 777 P. mirabilis	Dog	Alkaline	Alkaline	Alkaline throughout
P 100 P. mirabilis	Monkey	Alkaline	Alkaline	Alkaline throughout
S 926 P. mirabilis	Turkey	Alkaline	Alkaline	Alkaline throughout
Proteus OX19†		Alkaline	Alkaline	Alkaline throughout
Proteus OX K†		Alkaline	Alkaline	Alkaline throughout
Proteus vulgaris (1)	Human	Alkaline	Alkaline	Alkaline throughout
Proteus vulgaris (2)	Human	Alkaline	Alkaline	Alkaline throughout
Proteus vulgaris (3)	Human	Alkaline	Alkaline	Alkaline throughout

*American Type Culture Collection.
†Mich. Dept. Health Stock Culture Collection.

In Table II are recorded the reactions in urea media of thirty-eight strains of Salmonella obtained also from Mr. C. W. Darby. All strains came originally from Dr. P. R. Edwards of the University of Kentucky. Since these organisms had undergone several transfers since their initial typing, their Salmonella characteristics were checked by us. None of the organisms fermented lactose or sucrose, produced indol or liquefied gelatin. All were gram-negative and motile.

In Table III are recorded the reactions in urea media of thirty-seven Salmonella strains obtained from the stock culture collection of the Michigan Department of Health. Many of the organisms were identified by Dr. P. R. Edwards; the identity of the others was vouched for by the curator of stock cultures, Dr. Janet Bourn. All of the cultures were tested, however, for fermentation of lactose and sucrose, production of indol and liquefaction of gelatin,

gram staining qualities and motility. All of the organisms were Salmonella-like in their reactions.

In Table I it will be noted that all Proteus strains show evidence of decomposing urea with the exception of *Proteus ichthyosmius*. This organism produced a neutral reaction in Formula 1 and 2 and an alkaline slant surface in Formula 3, Ashworth's medium. Other Proteus organisms produced a deep blue color throughout Ashworth's medium.

TABLE II

REACTION OF THIRTY-EIGHT SALMONELLA STRAINS IN UREA MEDIA

CULTURE NO.	GROUP?	SPECIES	FORMULA 1 (RUSTIGIAN AND STUART)	FORMULA 2	FORMULA 3 (ASHWORTH'S)
284	A	*S. paratyphi* (15)	Neutral	Neutral	Alkaline surface
274	B	*S. typhi murium*, Var. Copenhagen (19500)	Neutral	Neutral	Alkaline surface
278	B	*S. bredeney* (1051)	Neutral	Neutral	Alkaline surface
281	B	*S. schottmuelleri* (A.M.S.)	Neutral	Neutral	Alkaline surface
282	B	*S. abortivoequina* (WH_2)	Neutral	Neutral	Alkaline surface
283	B	*S. derby* (1729)	Neutral	Neutral	Alkaline surface
285	B	*S. typhi murium* (M. 191871)	Neutral	Neutral	Alkaline surface
287	B	*S. california* (cal. 55)	Neutral	Neutral	Alkaline surface
375	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
376	B	*S. schottmuelleri*, Var. Odense	Neutral	Neutral	Alkaline surface
377	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
378	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
380	B	*S. heidelberg*	Neutral	Neutral	Alkaline surface
275	C	*S. muenchen* (425)	Neutral	Neutral	Alkaline surface
276	C	*S. cholerae suis* (1348)	Neutral	Neutral	Alkaline surface
279	C	*S. cholerae suis* Var. Kunzendorf (1713)	Neutral	Neutral	Alkaline surface
286	C	*S. newport* (197)	Neutral	Neutral	Alkaline surface
296	C	*S. barielly* (K.L.)	Neutral	Neutral	Alkaline surface
290	D	*S. gallinarum*	Neutral	Neutral	Alkaline surface
292	D	*S. enteritidis* (205)	Neutral	Neutral	Neutral throughout
352	D	*S. rostock*	Neutral	Neutral	Alkaline surface
353	D	*S. moscow*	Neutral	Neutral	Neutral throughout
354	D	*S. blegdam*	Neutral	Neutral	Alkaline surface
355	D	*S. berta*	Neutral	Neutral	Alkaline surface
289	E	*S. senftenberg* (5765-1)	Neutral	Neutral	Alkaline surface
293	E	*S. anatum* (M.C.M.1)	Neutral	Neutral	Alkaline surface
294	E	*S. new brunswick*	Neutral	Neutral	Alkaline surface
295	E	*S. give* (316)	Neutral	Neutral	Alkaline surface
372	E	*S. meleagridis*	Neutral	Neutral	Alkaline surface
373	E	*S. give*	Neutral	Neutral	Alkaline surface
288	Additional	*S. kentucky* (18)	Neutral	Neutral	Alkaline surface
291	Additional	*S. worthington* (Minn. 39)	Neutral	Neutral	Alkaline surface
358	Additional	*S. carrau*	Neutral	Neutral	Alkaline surface
298	Additional	*S. minnesota* (Minn. 2)	Neutral	Neutral	Alkaline surface
359	Additional	*S. onderstepoort*	Neutral	Neutral	Alkaline surface
356	Additional	*S. aberdeen*	Neutral	Neutral	Alkaline surface
357	Additional	*S. poona*	Neutral	Neutral	Alkaline surface

It was conjectured that the reaction of *P. ichthyosmius* in Ashworth's medium was due to the breakdown of nitrogenous substances in the meat extract component. All of the Proteus strains were therefore inoculated onto Ashworth's medium minus urea and were found with no exceptions to produce

alkaline slant surfaces but no reaction in the butt. Uninoculated controls were unchanged by incubator temperature.

The possibility of misinterpretation of reactions on this medium was undoubtedly realized by Ashworth[4] who cautioned that only the full range of brom thymol blue (on the alkaline side) denotes a positive result.

TABLE III

REACTION OF THIRTY-SEVEN SALMONELLA STRAINS IN UREA MEDIA

CULTURE NO.	GROUP	SPECIES	FORMULA 1 (RUSTIGIAN AND STUART)	FORMULA 2	FORMULA 3 (ASHWORTH'S)
501	A	*S. paratyphi*	Neutral	Neutral	Neutral throughout
502	A	*S. paratyphi*	Neutral	Neutral	Neutral throughout
504	A	*S. paratyphi*	Neutral	Neutral	Neutral throughout
503	A	*S. paratyphi*	Neutral	Neutral	Neutral throughout
564	B	*S. derby*	Neutral	Neutral	Alkaline surface
554	B	*S. san diego*	Neutral	Neutral	Alkaline surface
571	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
569	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
557	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
556	B	*S. san diego*	Neutral	Neutral	Alkaline surface
555	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
560	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
563	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
562	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
561	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
544	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
558	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
559	B	*S. schottmuelleri*	Neutral	Neutral	Neutral throughout
543	B	*S. typhi murium*	Neutral	Neutral	Alkaline surface
509	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
507	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
506	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
508	B	*S. schottmuelleri*	Neutral	Neutral	Alkaline surface
568	C	*S. newport*	Neutral	Neutral	Alkaline surface
565	C	*S. oranienburg*	Neutral	Neutral	Alkaline surface
570	C	*S. newport*	Neutral	Neutral	Alkaline surface
572	C	*S. oranienburg*	Neutral	Neutral	Alkaline surface
549	C	*S. newport*	Neutral	Neutral	Alkaline surface
546	C	*S. choleraesuis*	Neutral	Neutral	Alkaline surface
545	C	*S. choleraesuis*	Neutral	Neutral	Neutral throughout
550	C	*S. choleraesuis*	Neutral	Neutral	Alkaline surface
553	C	*S. bareilly*	Neutral	Neutral	Alkaline surface
552	C	*S. newport*	Neutral	Neutral	Alkaline surface
567	D	*S. panama*	Neutral	Neutral	Alkaline surface
547	D	*S. enteritidis*	Neutral	Neutral	Alkaline surface
542	D	*S. enteritidis*	Neutral	Neutral	Alkaline surface
551	E	*S. new brunswick*	Neutral	Neutral	Alkaline surface

In the media of Formulae 1 and 2 none of the seventy-five Salmonella cultures listed in Tables II and III gave any evidence of attacking urea. In Ashworth's medium none of the Salmonella cultures gave the deep blue coloration throughout the slant which characterized the growth of Proteus strains on this medium. However, 67 strains produced an alkaline surface. It was considered that these organisms, like *Proteus ichthyosmius*, were incapable of decomposing urea but did produce an alkaline reaction from the breakdown of the meat extract of Ashworth's medium. When the same Salmonella strains which produced an alkaline surface on the urea medium were grown on Ashworth's medium minus urea, they again produced alkali on the surface of the slope.

Rustigian and Stuart[2] tested the reaction of Proteus strains in their medium with and without urea and established that alkalinity resulted only when urea was present. Formula 2, with and without urea, was tested by us with Proteus and Salmonella cultures. An alkaline reaction resulted from the growth of Proteus only when urea was present; no change in the pH of the medium was produced by growth of Salmonella cultures whether or not urea was present.

DISCUSSION

The reactions of thirty Proteus and seventy-five Salmonella strains in urea media were unusually clear-cut. Only *Proteus ichthyosmius* of the thirty Proteus strains failed to decompose urea. None of the Salmonella cultures showed evidence of decomposing urea.

It is true that all of the organisms tested by us were stock cultures or had lived for several generations on artificial media. In addition to these, approximately fifty newly isolated Salmonella strains have been tested for urea decomposition in the laboratories of the Michigan Department of Health during the last eight months. None of the Salmonella cultures attacked urea.

It will not be surprising if one or more Salmonella of the 107 species listed by Kauffmann[7] are found capable of attacking urea. Some strains of *S. Eastbourne*, it will be remembered, vary from the Salmonella characteristics by producing indol; three species of Salmonella liquefy gelatin and a variant of *S. anatum* has been described which ferments lactose. However, the results in this paper indicate that urea-decomposing strains of Salmonella will rarely, if ever, be encountered.

Of the three urea media used our inclination is in favor of the liquid medium of Rustigian and Stuart, or Formula 2. Ashworth's solid medium, while convenient to use, may very well give misleading results to one who is unfamiliar with its reactions with and without urea.

SUMMARY

Of thirty strains of Proteus, *Proteus ichthyosmius* only failed to decompose urea.

None of seventy-five strains of Salmonella showed evidence of attacking urea.

A test for urease activity is recommended as a routine procedure for separation of Proteus and Salmonella organisms.

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CLINICAL CHEMISTRY

THE CEPHALIN FLOCCULATION TEST IN JAUNDICE*

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IN 1938 and 1939 Hanger[1, 2] reported a new laboratory test employed as a diagnostic measure in jaundice of various types. It was an empirical reaction, of incompletely understood mechanism, which depended on the flocculation of a cephalin-cholesterol emulsion in the presence of the serum of patients with active parenchymal disease of the liver. In a series of over 900 normal individuals there was only one weakly positive reaction, while in catarrhal jaundice and acute hepatitis it was found to be positive at the height of the disease. The reaction in obstructive jaundice was negative or only very weakly positive, the latter occurring in cases of prolonged obstruction and/or acute associated hepatitis. The test was found to parallel the activity of the pathologic process in cirrhosis. Hemolytic jaundice gave negative results. However, Hanger[1] listed a number of miscellaneous conditions with or without jaundice, including such diseases as subacute bacterial endocarditis, various types of pneumonia, nephritis, and other infections in which the test was positive in a goodly proportion of instances. Nevertheless, if the clinical picture, corroborative laboratory data, and pathologic findings were carefully studied, and particularly if the test was confined to jaundiced individuals, there was a good indication that the cephalin flocculation reaction was a reliable index of the degree and type of active parenchymal disease of the liver.

In 1940 Hanger and Gutman[3] published the clinical, chemical, and pathologic findings in cases of icterus following arsphenamine therapy. These observers had previously noted that in a minority of patients with postarsphenamine jaundice the cephalin flocculation test gave negative results and that the serum phosphatase activity was elevated, indicating an obstructive intrahepatic type of icterus rather than the usual parenchymatous type. This was confirmed by pathologic examination. It was on the basis of these studies that the cephalin flocculation test was found to have selective value in differentiating obstructive from nonobstructive jaundice.

Since then, several observers have reported their results with the flocculation test, and although their findings occasionally vary from those of Hanger, they all accept the test as a dependable aid in the differential diagnosis of jaundiced conditions. Pohle and Stewart[4] found in a series of 141 patients with hepatic or biliary tract disease that the reaction was positive in varying degrees in most instances. However, they regarded a one- or two-plus reaction as positive. They were impressed with the sensitivity of the reaction in detecting degrees of liver damage rather than its selectivity in distinguishing

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obstructive from nonobstructive jaundice. In their hands false positive reactions were rare. Rosenberg[5] also emphasized the delicacy of the test, for he considered any positive reaction (even as slight as plus-minus) as indicative of liver disease. In employing the procedure as a means of separating obstructive from parenchymatous jaundice, he based his conclusion on the degree of flocculation, rather than on its presence or absence. Nadler and Butler[6] agreed in general with the observations of Hanger. Of particular interest is their finding that four cases of jaundice following arsphenamine therapy all gave negative reactions, thereby confirming the observations of Hanger and Gutman. They employed a cephalin which was obtained from the same source as that used in the present study and suggested that the reagent used by Pohle and Stewart was too sensitive.

Mateer, Baltz, Marion, Hollands, and Yagle[7] reported an extensive comparative laboratory evaluation of hepatic and biliary tract disease which included the cephalin cholesterol flocculation reaction. They noted that the test was at once specific and sensitive and recommended it without reservation. However, since their cases are not grouped from the standpoint of "medical" versus "surgical" jaundice, their results are not strictly comparable to those included in this paper. Nevertheless, they pointed out that although Hanger held that "unripened" cephalin (i.e., that which is not allowed to oxidize by lengthy exposure to air) is more sensitive and more liable to give false positive reactions, the reverse held true in their experiences. Specifically, in 40 normal controls there were 5 one-plus reactions after forty-eight hours with the "unripened" material, while the use of the "ripened" substance gave several times as many positive results, some even two-plus reactions. A more recent report[8] relates the relative value of the commercial substance made by the Difco Company of Detroit (that used in this work), namely, that a one-plus reaction per se had no positive significance.

The present study includes 154 cases of jaundice observed on the wards of the Mount Sinai Hospital from October, 1941 to April, 1943. They have been grouped under the headings of obstructive and hepatocellular jaundice, with particular reference to the differentiation of medical and surgical conditions. The sensitivity of the cephalin used (Difco product) coincided with that in Dr. Hanger's laboratory, and the methods employed in the performance of the test were in accord with his directions. The results of the reaction have been designated as 0, 1 plus, 2 plus, 3 plus, and 4 plus, and readings were made after twenty-four and forty-eight hours. Although the 24-hour reading was considered more sensitive, the interpretation of the test was based upon the 48-hour reading, because this was found more clear-cut and reliable. For the sake of reliability and in view of other clinical, chemical, and pathologic findings, the cases giving a 1-plus reaction were interpreted as negative. Dr. Hanger had suggested that a 1-plus reaction had no definite significance in relation to parenchymatous disease, unless this was noted during recovery or in cirrhosis. In a large number of cases the test was done several times in the course of the disease, and some of the repeated determinations indicate the variation correlated with the dynamics of the pathologic process. The proved cases were confirmed by post-mortem examination or surgical exploration.

The results are summarized in Tables I and II.

TABLE I

OBSTRUCTIVE JAUNDICE

		NEGATIVE		POSITIVE		
CALCULOUS OBSTRUCTION		0	+	++	+++	++++
(Common Duct Stone)						
Number Cases	33	22	8	3	0	0
Proved	25	16	6	3	0	0
CARCINOMATOUS OBSTRUCTION						
(Common Duct or Pancreas)						
Number Cases	19	15	1	2	1	0
Proved	16	13	0	2	1	0
CICATRICIAL OBSTRUCTION						
Number Cases	11	5	1	0	3	2
Proved	8	3	1	0	2	2
LYMPH NODE OBSTRUCTION						
Number Cases	5	2	3	0	0	0
Proved	2	1	1	0	0	0
ACUTE CHOLECYSTITIS AND JAUNDICE						
(No Obstructive Factor Demonstrated)						
Number Cases	5	3	1	1	0	0
Proved	5	3	1	1	0	0
CHRONIC CHOLECYSTITIS AND JAUNDICE						
(No Obstructive Factor Demonstrated)						
Number Cases	2	1	0	1	0	0
Proved	2	1	0	1	0	0
TOTAL CASES	75	48	14	7	4	2
PROVED	58	37	9	7	3	2

TABLE II

HEPATOCELLULAR JAUNDICE

		NEGATIVE		POSITIVE		
PARENCHYMATOUS		0	+	++	+++	++++
(Toxic Hepatitis, Catarrhal Jaundice)						
Number Cases	58	17	3	2	10	26
Proved	8	3	1	1	0	3
NEOARSPHENAMINE						
Number Cases	4	3	1	0	0	0
Proved	0	0	0	0	0	0
CIRRHOSIS						
Number Cases	17	4	1	5	2	5
Proved	8	2	0	2	1	3
TOTAL CASES	79	24	5	7	12	31
PROVED	16	5	1	3	1	6

DISCUSSION

In the cases of obstructive jaundice, the cephalin flocculation test showed an accuracy of 83 per cent. It would appear that the problem of differential diagnosis of "medical" vs. "surgical" jaundice was nearer a solution by this reaction. Unfortunately, as will be shown later, a fairly large number of instances of parenchymatous jaundice gave negative reactions. At any rate, our interpretation of the 1-plus reaction as negative appeared to be well grounded.

In cases of "infectious" hepatitis the reaction was positive in about two-thirds of the cases. Although the sensitivity of the test does not appear to be remarkable, its reliability when positive is great, for whenever a 3-plus or 4-plus reaction occurred, the diagnosis of parenchymatous liver disease was confirmed

by chemical or pathologic data. Only rarely did a case of obstructive jaundice give a positive reaction, and then only weakly positive such as 2 plus.

The type of toxic reaction to organic arsenical therapy described by Hanger and Gutman,[3] i.e., an intrahepatic obstructive icterus, was encountered in 4 cases. All were in individuals who received only two or three doses of the arsenical (in these instances, neoarsphenamine). The onset of icterus was sudden and in 2 cases the jaundice was prolonged. It should be noted that in Nadler and Butler's[6] cases as well, the flocculation test was negative in all instances.

In cirrhosis, the cases exhibiting evidence of active liver disease as evidenced by jaundice, as well as other stigmata of hepatic insufficiency yielded positive reactions in only half of the cases. In an additional three cases not tabulated because jaundice was absent, two were 0, and one was 1 plus.

SUMMARY AND CONCLUSIONS

1. By means of the cephalin flocculation test 164 assorted cases of jaundice were studied over a 22-month period, with interest directed toward differentiating obstructive from nonobstructive icterus.

2. Of 75 cases of obstructive jaundice, 62 were "negative."

3. Of 58 cases of hepatitis, 20 were negative and 38 were positive.

4. Of 4 cases of postarsenical icterus, all were negative.

5. Of 17 cases of cirrhosis with jaundice, 12 were positive.

6. In 3 cases of hemolytic jaundice, the reaction was negative in 2, positive in 1.

7. In our laboratory, the cephalin flocculation test reached its highest degree of accuracy in the obstructive jaundice group, but paradoxically was more reliable in the hepatitis group because false positives were a rarity.

8. To our mind the 1-plus reaction is of no significance in distinguishing obstructive from nonobstructive jaundice.

9. In general, the cephalin flocculation test may offer assistance in the differential diagnosis of jaundice. However, in our hands its accuracy did not concur with the results published by Hanger.

We wish to express our gratitude to the Difco Company of Detroit for their supplies of cephalin and to the Eli Lilly Company of Indianapolis for merthiolate.

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OBSERVATIONS ON THE INGESTION OF METHYL CELLULOSE AND ETHYL CELLULOSE BY RATS*

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BECAUSE of the potential usefulness of methyl and ethyl cellulose as thickening and binding agents in the pharmaceutic and food industries, the possibility that they might have deleterious effects was investigated by feeding them to rats over a considerable period of time.

Methyl cellulose[1] is a white, fibrous, cellulose ether, soluble in cold water but insoluble in hot water and in most organic solvents. Heating causes most aqueous solutions of methyl cellulose to coagulate, but cooling and stirring restores them to their original smoothness and fluidity. Solutions of the compound are colorless, odorless, and tasteless. Ethyl cellulose,[2] also a cellulose ether, is insoluble in water but soluble in most organic solvents. Its solutions are colorless and odorless.

Acknowledgment is made to the Dow Chemical Company for the financial support of the investigation and for the supply of the materials in the form of Methocel (Lot M-368, viscosity 1500 centipoise) and Ethocel (Lot 2467, viscosity 20 centipoise).

GENERAL EXPERIMENTAL PROCEDURES

Young albino rats, male nad female, bred in this laboratory and all of approximately the same age and weight, were divided into 3 groups of 80, to be fed the two cellulose ethers and the control diet, respectively. They were caged in groups of twenty. The food was supplied in baskets of wire screening, and the drinking water, in bottles fitted with glass tubes, the quantities consumed being recorded weekly. Experiments were started in July and terminated in the following March, at which time a representative number of animals from each group was killed and examined for gross and microscopic tissue changes.

PREPARATION OF FOOD AND WATER

Eighty grams of methyl cellulose were wet with hot water, cooled to room temperature, and stirred until nearly smooth; then one liter of cold water was added, and after the mixture had been kept about twenty-four hours in the refrigerator, the whole was diluted to 1500 c.c. with water, mixed, and added to 10 kg. of Purina Fox Chow pellets. Thus 10 Gm. of the dried food contained 80 mg. of methyl cellulose (8,000 parts per million). The drinking water was prepared so as to contain 10 Gm. of methyl cellulose in one liter of water (10,000 parts per million).

Ethyl cellulose (120 Gm.) was dissolved in 3 liters of 95 per cent ethyl alcohol and added to 10 kg. of Purina Fox Chow. After the alcoholic solution had

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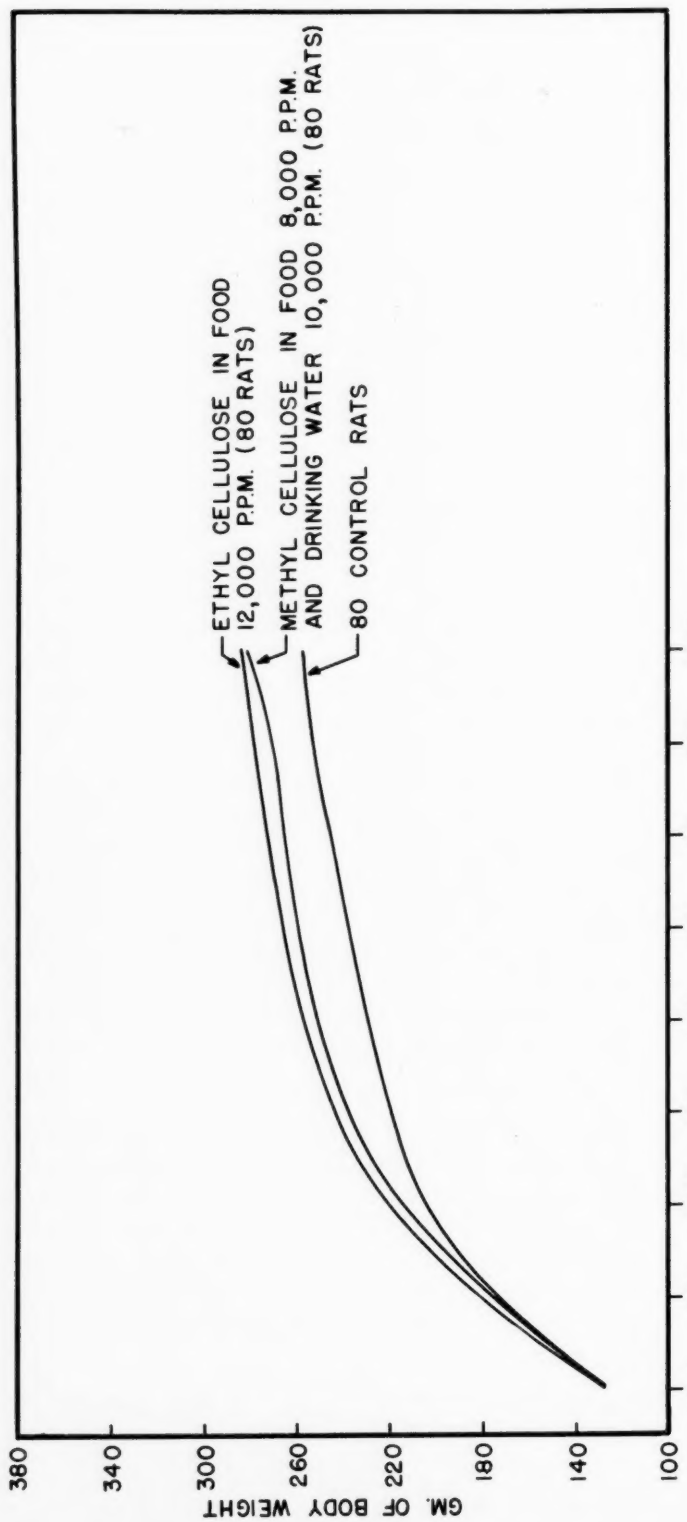
AVERAGE WEIGHTS OF RATS FED
METHYL CELLULOSE OR ETHYL CELLULOSE
ETHYL CELLULOSE IN FOOD
12,000 P.P.M. (80 RATS)
METHYL CELLULOSE IN FOOD 8,000 P.P.M.
AND DRINKING WATER 10,000 P.P.M. (80 RATS)
80 CONTROL RATS
GM. OF BODY WEIGHT
380
340
300
260
220
180
140
100
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.

Fig. 1.

soaked in, the pellets were heated for two days at 70° C. in order to expel the solvent. (Analyses proved that the alcohol had been expelled except for insignificant traces.) Ten grams of this food contained 120 mg. of ethyl cellulose (12,000 parts per million).

RESULTS

Group 1.—The 80 rats in this group were given both food and water containing methyl cellulose. The consumption of food varied from 13 to 19 Gm. per day, and the daily intake of water ranged from 25 to 34 c.c. so that the average total daily intake of methyl cellulose was about 436 mg. per rat, 125 mg. being ingested with the food and 311 mg. in the drinking water.

Group 2.—The 80 rats in this group ingested from 14 to 18 Gm. of food per day, the daily intake of ethyl cellulose being about 182 mg. per rat. These animals had free access to uncontaminated drinking water.

Group 3.—The consumption of food and water by the 80 control animals varied within the limits recorded in the case of Groups 1 and 2 above.

Fig. 1 portrays the weight changes that occurred in each group as a whole. The differences among the three groups in this respect were manifestly insignificant. No other evidences of differences in the behavior or well-being of the animals in the several groups were noted.

SUMMARY AND CONCLUSIONS

Eighty rats were given methyl cellulose in their food and drinking water over a period of eight months, and the same number were given ethyl cellulose in their food for a corresponding period. The daily ingestion of quantities of methyl cellulose amounting to about 0.44 Gm. per rat or of ethyl cellulose amounting to about 0.18 Gm. per rat produced no signs of illness. The consumption of food and water as well as the gain in weight by these animals was normal. No evidence was found of significant gross or microscopic abnormalities in the tissues of the animals. It is concluded that the feeding of methyl and ethyl cellulose in these concentrations is harmless to rats.

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LABORATORY METHODS

GENERAL

AN INK-WRITING FINGER PLETHYSMOGRAPH*

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VARIOUS forms of finger plethysmograph are in use, employing photographic methods or using photoelectric cells. At least one form of ink-writing plethysmograph has been described,[1] though the data concerning its construction are not available. The present method has the advantage of being relatively inexpensive, has proved exceedingly simple and reliable to use, and can be made by anyone with a moderate knowledge of electronics. The advantage of an ink record of volume changes is obvious, since it makes possible observations over prolonged periods of time and provides a tracing which can be examined immediately, without the trouble and expense of developing it, as with the photographic method. The apparatus herein described also has the advantage that, since the plethysmograph chamber on the finger is connected to the recording unit only by fine flexible leads, slight movements of the hand do not tend to displace the plethysmograph relative to the finger. The plethysmograph is free in other words to move more or less as the hand moves.

The metal plethysmograph chamber (Fig. 1) is attached to a metal plate shaped to fit the palm and loosely bound to the palm by adhesive or spring clips at the edges. The latter method avoids obstruction of the venous return from the fingers. The distal end of the plethysmograph chamber is closed off with a slack condom rubber diaphragm. At the distal end of the chamber a bracket extends at right angles. This is made of metal, and being attached to the metal chamber is therefore at body or ground potential. Several parallel metal plates (4 to 8) are attached at right angles to this bracket. A moving arm is suspended on an insulator at the outer end of this bracket and is fitted also with parallel metal plates, which slide between the metal plates of the bracket without touching them, thus forming a variable condenser. The moving arm and its plates are made of aluminum foil (#35 gauge) for lightness, weighing only 4.0 Gm. Strength is obtained by flanging the edges where necessary. Movement is imparted to the movable arm by the condom diaphragm through a connecting pin. A gentle spring holds the pin constantly in contact with the diaphragm.

The finger is inserted into the plethysmograph with the "adjustment capsule" removed (cf. Fig. 1). An airtight seal is obtained simply by pressing

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vaseline between the edge of the plethysmograph and the skin. The "adjustment capsule" is then slipped onto the "stem." The adjustment screw is then tightened, thus depressing the thick rubber diaphragm beneath it, until the condom diaphragm is displaced outward to bring it into the mid-zone of its movement range. The two lead wires are then connected and the record may be taken. The preparations for a tracing take about a minute.

A calibrator is built into the plethysmograph. It consists simply of a thick rubber diaphragm, which is displaced by movement of a lever. It permits quick calibration of each tracing to be made. The calibrator must first be standardized against a known standard, the one in use having a displacement of .06 c.c.

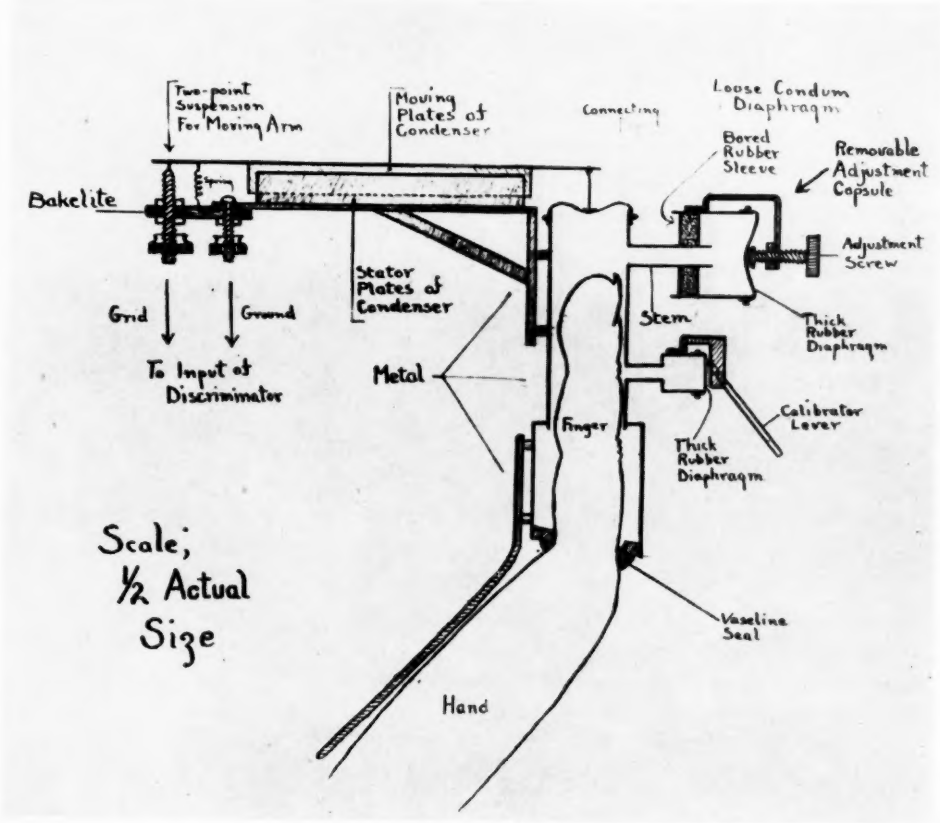
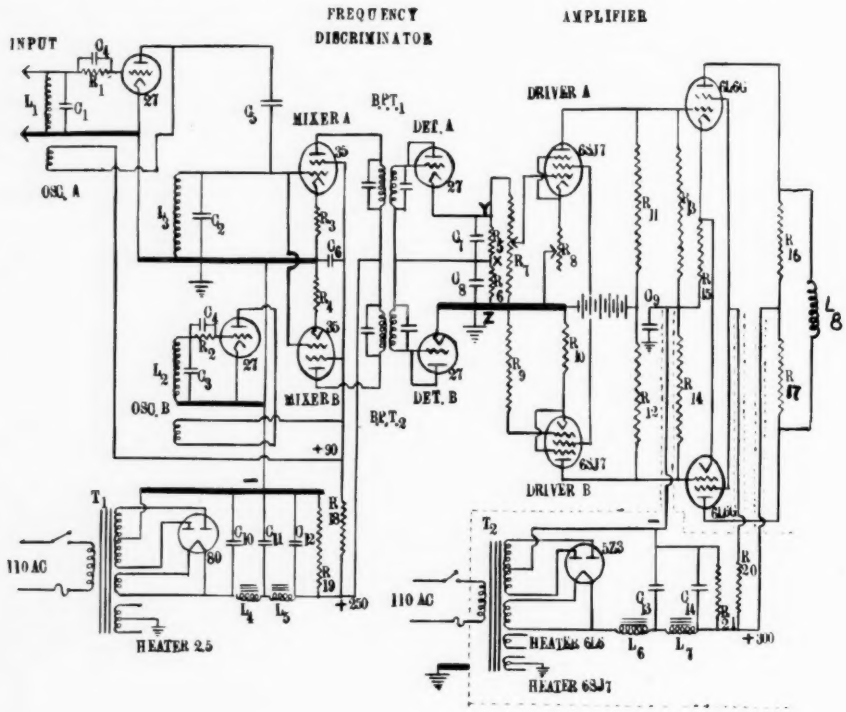


Fig. 1.—Scale diagram of finger plethysmograph.

The changes in finger volume are converted into changes of voltage which operate the recorder. The electrical circuit consists of a frequency discriminator which converts changes in frequency in an oscillator to voltage changes, which are amplified. Two oscillator circuits, A and B (Fig. 2), are tuned to beat against each other. The resulting beat frequency is fed into two mixer tubes in parallel. Mixer tube A passes its output into a band-pass transformer tuned to 175 kilocycles, and mixer tube B passes its output into a band-pass transformer tuned to 172.5 kilocycles. When the frequency of oscillator A is

tuned by its condenser C_1 to beat with oscillator B to produce a beat of 175 kilocycles, the maximum flow of current is produced through detector circuit A, and a maximum voltage drop is produced across resistor R_5. At this frequency the band-pass circuit B does not respond, and no current flows through detector circuit B. Consequently no potential difference is developed across



C_1, C_2, .0005 mfd variable. C_3, .00035 mfd mica. C_6, .5 mfd. C_9, 1 to 4 mfd.
C_4, C_5, C_7, C_8 .00025 mfd. C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, 8 mfd. electrolytic, 450 volts wkg.
R_1, R_2, 2 to 5 meg. R_3, R_4, 350 ohms. R_7, 500,000 ohms pot. R_8, 3000 ohms wire wound
R_5, R_6, R_{11}, R_{12}, R_{18}, 250,000 ohms. R_{10}, 1700 ohms. R_9, R_{13}, R_{14}, 500,000 ohms.
R_{15}, 240 ohms. R_{16}, R_{17}, 2000 ohms, 20 watts. R_{20}, 5000 ohms.
T_1, T_2, Power supply transformers.
B.P.T.$_1$, Band-pass transformer, 175 kc. B.P.T.$_2$, Band-pass transformer, 172.5 kc.
L_1, L_2, Tuning and oscillator coils, 540–1500 kc., wound on a common form.
L_3, Tuning coil. 540–1500 kc. L_4, L_5, 20 Henries, 100 ma. L_6, 10 Henries, 200 ma.
L_7, 1500 ohm field coil of recorder, 20 watts. L_8, Moving coil of recorder, 200 ohms.
Note; Power supply of output stage is all above ground and must be carefully shielded.

Fig. 2.—Schematic diagram of frequency discriminator and amplifier.

resistor R_6, so that points X and Z are at the same potential. Since there is a voltage drop across resistor R_5, point Y is positive with respect to point X and therefore also with respect to point Z. As a result the control grid of driver stage A becomes positive with respect to ground. As the potential of the control grid of driver stage B is not changed, the direct-coupled push-pull amplifier is thrown off balance, and moving coil L_8 connecting the two plates of the 6L6G output tubes is displaced. If oscillator A is tuned by its condenser to produce

a beat of 172.5 kilocycles, the band-pass circuit B responds to its maximum, and a maximum voltage drop occurs this time across R_6, making X negative to Z. Since no response is induced in band-pass circuit A at this frequency, no voltage drop occurs across R_5, and Y and X remain at the same potential. Y is thus negative with respect to Z, in consequence of which the control grid of driver A becomes negative with respect to ground, while the potential of control grid of driver B remains unchanged. The push-pull direct-coupled amplifier is put off balance in the opposite direction, and moving coil L_8 is moved in the opposite direction. When oscillator A is tuned to give a beat midway between 172.5 and 175 kilocycles, both band-pass circuits respond equally and an equal voltage drop occurs across R_5 and R_6. Y and Z are then at the same potential so that the control grid of driver A remains at ground potential, and the amplifier remains balanced. From this position of balance any change of frequency of oscillator A will change the beat frequency so that more current is induced in either band-pass circuit A or B.

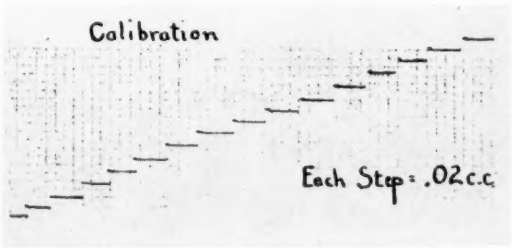


Fig. 3.—Record of successive increments of .02 c.c. into the plethysmograph from a pipette, showing the linearity of the response. Greater sensitivity can be obtained when desired.

When the plethysmograph is connected to the discriminator, the fixed plates of its variable condenser are connected to ground and the moving plates to the grid of oscillator A. By means of variable condenser C_1 the beat frequency is adjusted until the writing point connected with moving coil L_8 is brought to the mid-position. The correct setting of the condenser is easily found, for by turning it either way from the point of central adjustment, the writing lever should move an equal amount, first to one side, then to the other, as the movement of the condenser is reversed. Any variation of the position of the moving plates of the plethysmograph condenser, induced by changes of finger volume, causes a change in the frequency of oscillator A and therefore of the beat frequency and thus displaces the recording pointer.

In order to reduce undesirable interaction between the two oscillators, oscillator A is capacitatively coupled to the mixers and oscillator B is inductively coupled. The use of two mixers in parallel simplifies alignment of the band-pass circuits since they are then independent. Each circuit can be aligned separately without upsetting the alignment of the other. A separate high-voltage supply is used for the discriminator circuit. Condenser C_2 is used to tune the mixers to give the maximum response. The variable grid, bias on only one of the 6SJ7 tubes, permits the voltage at the plates of the 6L6G tubes to be equalized. This is important, since with the direct coupling of the drivers to

the output tubes, slight inequalities in function of the driver tubes might cause serious imbalance in the output tubes. It must be noted also that the plate supply of the output stage is all above ground and requires special care in wiring and mounting. Since the heaters of the 6L6G tubes are also above ground, this heater winding is not center tapped. Condenser C_9 is to eliminate an A.C. component in the output. It should be a paper condenser to avoid leakage through it from the plate supply of the drivers which would tend to make the grids of the output tubes positive. The recording pointer is activated by a moving coil, suspended by springs in a dense magnetic field. The battery plate supply of the driver stage may be replaced by another power source. A potentiometer is used in the grid of the driver stage to control the gain of the amplifier. In order to vary the sensitivity of the variable condenser on the plethysmograph and to make it of service whether small or large changes of volume might occur, some of the plates of the stator can be removed in a moment from spring clips that hold them in place.

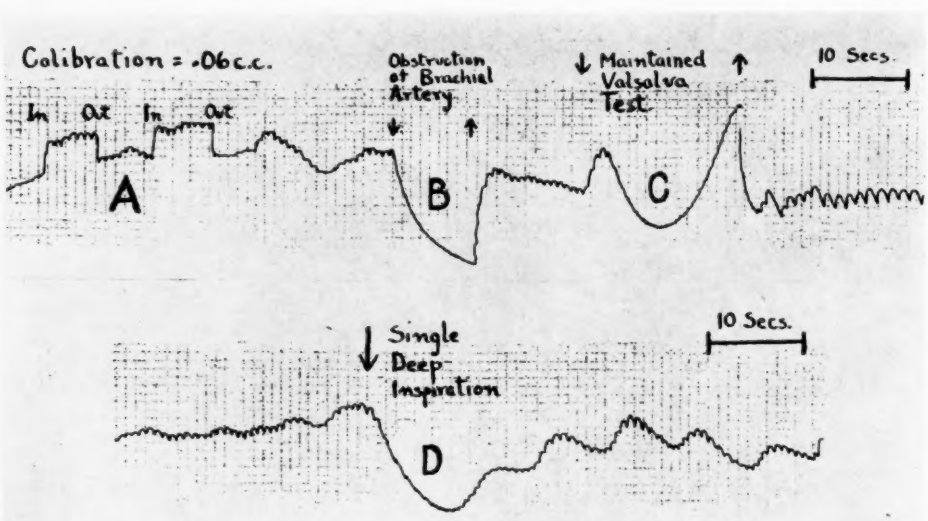


Fig. 4.—Tracings taken with the finger plethysmograph.

A, Calibration with .06 c.c. displacement, using the calibrator built into the plethysmograph.

B, Passive vasoconstriction due to obstruction (by pressure) of the brachial artery. Prompt return of volume on release of pressure.

C, Biphasic response with maintained forced expiration against the closed glottis. Prompt return of volume when forced expiration stopped, followed by large pulse waves.

D, Active vasoconstriction due to a single deep inspiration. Subsequent undulations due to respiratory movements.

Vasoconstriction or reduced finger volume causes downward movement of the recorder. Vasodilatation or increased finger volume causes upward movement of the recorder.

A calibration of the plethysmograph made by injecting successive volumes of 0.02 c.c. air into the chamber is shown in Fig. 3, indicating the linearity of the response.

It should be pointed out that the type of variable condenser on the plethysmograph does not lend itself to rapid volume changes. Frequency calibration curves have not been done, since the apparatus is designed primarily to record the slow volume changes of a finger due to changes of vascular control.

It is possible that some distortion would occur in the recording of the pulse curves, especially in the sharply rising phase. But such distortion, if present, would not lessen the reliability of records of the slow volume changes.

The actual apparatus in use was made from odd materials at hand. More recent types of tubes, etc., could profitably be substituted if available. Maximum voltage variations between Y and Z (Fig. 2) were about 20 volts. Maximum excursion of the pointer is about 6 cm. with a lever ratio of approximately 6 to 1.

Typical records of finger volume changes are shown in Fig. 4.

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MACHINE-ROLLED STOPPERS FOR CULTURE TUBES

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MUCH of the loss of materials and of the labor required to make stoppers for culture tubes may be avoided if machine-rolled stoppers that can be used repeatedly are made by a lathe such as herein described.

The tongue of the stopper-spindle (1), two inches long, tapers from one-eighth of an inch at the shoulder to one-sixteenth of an inch at the tip. A second spindle for making hollow stoppers for use with pipettes is eleven sixty-fourths and ten sixty-fourths of an inch in diameter at the shoulder and tip respectively. The spindles, driven by a one-fortieth horsepower motor, are mounted on the motor shaft by means of a work arbor and chuck (2). A 20-gauge steel channel with flared flanges comprises the stopper forming table (3). The right half of the channel web, raised an amount equivalent to one thickness of the sheet steel, forms the tapered end of the stopper. A slot is cut in the left-hand flange to permit the spindle to function. The forming table is secured with two machine screws (4), near the left side, to an inclined forming table support (5). Two setscrews (not visible), mounted from below at the right side of the forming table support, bear on the under side of the forming table and permit tapering the smaller end of the stopper to any desired taper.

The stopper's diameter is controlled by an adjustable mechanism mounted on the upper end of the forming table support (5). The mechanism consists of a short channel (6), two setscrews (7), two flat springs (8 and 9), and a spring support (10). The lower setscrew (only partially visible) presses against the lower side of spring (8), one end of which is fixed to the lower side of the spring support (10). The angle of the latter conforms with the angle of the forming table support. The upper setscrew (7) presses against the upper side of a slightly bent flat spring (9), one end of which is free but whose

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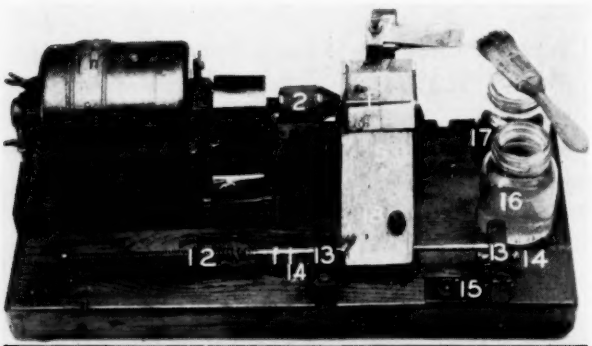

FIG. 1

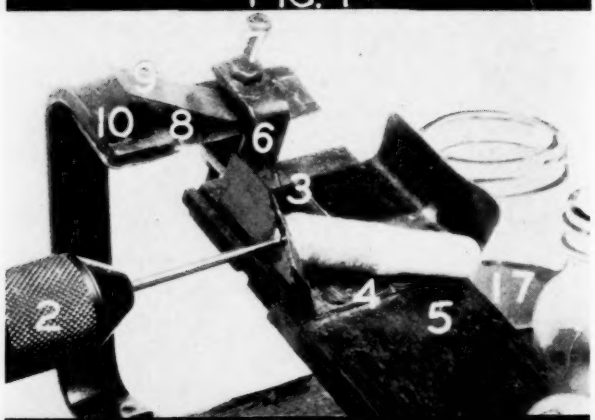

FIG. 2

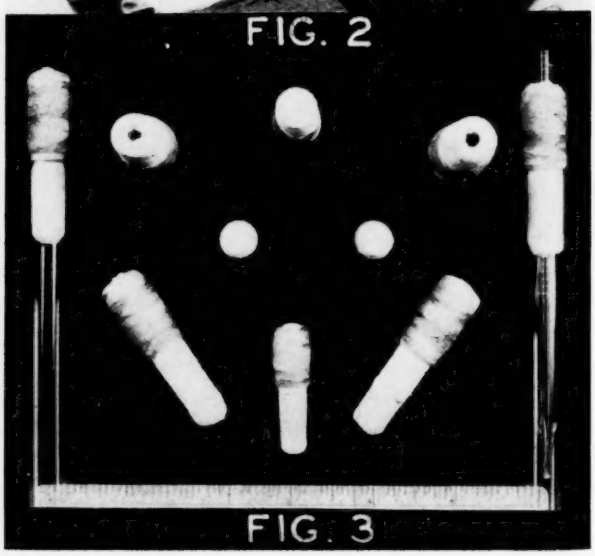

FIG. 3

other end is attached to the free end of spring (8). These two springs hold the forming table firmly against the spindle or cotton as the stopper is being rolled. Passing through, and tightly fitting into, the lower end of the forming table support runs a shaft (11), to whose left end is attached a coiled returner spring (12). Brass bearings mounted in walnut pillow blocks (13) support the shaft. Two adjusting studs (14) control the right and the left positions of the forming table support. A base, eight inches by sixteen inches, supports the entire mechanism.

The motor is started by means of the toggle switch (15), the spindle is moistened with water from (16), approximately the correct amount of cotton or other fibrous material is touched to the spindle, and the stopper is quickly rolled. The handle of the stopper may be painted or sprayed with a tinted plastic in solution (17), and the stopper may then be removed by pushing the forming table support to the right by means of post (18). Small stoppers may be made if a small-sized forming table is clamped to the right side of the larger forming table. Some stoppers made by the lathe are shown in Fig. 3. Stoppers have been made of absorbent and nonabsorbent cotton, fiber glass, and even steel wool. The latter two are only curiosities at the present time. The most satisfactory material yet used is a combustion-resisting DuPont cellulose acetate product known as 3-F Continuous Filament Batting. Being in an experimental stage, this material is not yet available commercially.

A NEW METHOD OF COUNTING PLASMODIA IN AVIAN MALARIA INFECTIONS*

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THE best known and probably most used method of counting plasmodia in the peripheral blood in avian malaria infections is that in which the result of the count is recorded in terms of plasmodia per 10,000 erythrocytes. One of the forms of this "standard" method consists in counting the plasmodia seen in 1,000 erythrocytes and simply multiplying by 10; one counts erythrocytes up to 1,000 "in the head" while simultaneously recording plasmodia with pencil marks on a pad; or one counts the plasmodia on a pad or "in the head" and records erythrocytes on a mechanical counter; or one counts the erythrocytes first, notes on the verniers of the mechanical stage the limits within which the count was made, and then goes back and counts the plasmodia within these limits. In any case the resulting plasmodial count is multiplied by 10 and recorded as organisms per 10,000. We have not been able to satisfy ourselves that our counts are notably accurate when made in any of the above ways.

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A variant of the "standard" method consists in averaging the counts of 5 fields and dividing this number into 1,000 to determine the number of fields in which plasmodia must be counted in order to record the organisms per 10,000 (after multiplying by 10, of course); or one may divide directly into 10,000 and then make an actual count of the resultant very large number of fields. Working as we do, with a 92x objective and a 10x eyepiece, we find fields ideal for counting (i.e., well filled but with the erythrocytes not touching) to contain 90 to 100 cells; this would require us to see only 10 or 11 fields in order to obtain our 1,000 cells; we think this too few, but on the other hand to examine the 100 to 110 fields, which would be required to complete the count of 10,000, would certainly not be practicable. Another reason for our inability to be entirely satisfied with this method is that it assumes an almost perfect uniformity of smearing, which we are not certain that we invariably achieve.

The only other variant of the "standard" method with which we are familiar is to apply in each separate instance a formula by which to determine the number of erythrocytes to be examined, if the plasmodial count is to have an error of no more than 10 per cent. Gingrich[1] states this formula as follows: $N = 45.494 \frac{I-P}{P}$ in which N = the number of erythrocytes to be counted, P = the number of parasites per sample unit, and I = the sample unit (10,000 erythrocytes). At the present time, when a very large number of smears must be examined daily in all laboratories, we feel obliged to think of this method as having only academic interest.

Several years ago the senior author[2] offered a method which differed entirely from the "standard" method or any of its variants: A fixed number of plasmodia were counted, and then the count was recorded in terms of the time required to make it. This method, which enables one very simply to record degrees of infection as +, 2+, 3+, 4+, and 5+, served us in a practical way for a time, but it failed to prove sufficiently delicate to reflect slight differences in the antimalarial activities of the newer compounds which are being studied nowadays. We have therefore devised, and have been using with satisfaction for more than a year (i.e., in many hundreds of infections), the method now to be described.

DESCRIPTION OF OUR NEW METHOD

Using a 92x oil immersion objective and a 10x eyepiece, we give the slide a hasty examination to find an area in which we can range through a good many fields without encountering excessively wide variations in the thickness of the smear. Then the count is made:

The method consists in counting the number of organisms seen in three minutes of a *standardized type of search*. One starts the stop watch and then looks at every erythrocyte in the field. This is important; the whole field is not just quickly scanned and the number of plasmodia present noted, but instead every erythrocyte is looked at and the number of plasmodia seen during the course of this examination of the erythrocytes is counted. Then one turns to the next field and continues to examine erythrocytes and count plasmodia this way until the three minutes have elapsed. The total number of plasmodia counted in the three-minute period is then recorded as the index of the infec-

tion on that day. The watch is not stopped as one shifts from field to field, but it is stopped when glancing at it to determine the amount of elapsed time.

Two variants of the above are employed to make the method applicable to all the situations arising. (a) A negative slide is treated just as is a positive one; i.e., all erythrocytets are looked at in all the fields which are viewed in the three-minute period, but if a plasmodium is not encountered, the slide is simply recorded as negative. (b) If, in the examination of an apparently negative slide, a plasmodium is finally encountered before the three minutes have elapsed, the watch is stopped and reset at zero, and one starts over on a fresh count of three minutes beginning with this organism as plasmodium 1. This latter situation arises almost exclusively only on the first day of infections, for practically always on the days thereafter there is a sufficient abundance of organisms so that one is quickly able to find a field containing at least 1 plasmodium with which to begin the count.

TABLE I

COURSE OF THE *P. cathemerium 3H2* INFECTIONS IN FIVE CANARIES DURING THE FIRST FIVE DAYS AS COUNTED BY THE "STANDARD" METHOD AND BY OUR NEW METHOD

DATES	OUR METHOD			"STANDARD" METHOD		
	COUNT	TIME REQUIRED	R.B.C. SEEN	COUNT	TIME REQUIRED	R.B.C. SEEN
Bird #975						
12-7	2	3′45″	2,841	1	4′45″	1,000
12-8	25	3′	2,290	6	4′50″	1,000
12-9	132	3′	2,258	91	4′30″	1,000
12-10	263	3′	2,279	195	4′30″	1,000
12-11	220	3′	2,187	125	4′30″	1,000
Bird #974						
12-8	3	3′45″	2,838	1	4′25″	1,000
12-9	25	3′	2,283	7	4′30″	1,000
12-10	89	3′	2,255	40	4′30″	1,000
12-11	203	3′	2,314	135	4′25″	1,000
12-12	153	3′	2,243	120	4′30″	1,000
Bird #548						
12-16	6	3′36″	2,743	0	5′	1,000
12-17	23	3′	2,231	8	4′30″	1,000
12-18	88	3′	2,187	49	4′30″	1,000
12-19	196	3′	2,267	113	4′30″	1,000
12-20	154	3′	2,261	95	4′30″	1,000
Bird #522						
12-16	3	4′10″	3,134	1	5′	1,000
12-17	27	3′	2,265	10	4′55″	1,000
12-18	77	3′	2,266	33	5′	1,000
12-19	156	3′	2,351	74	5′	1,000
12-20	93	3′	2,257	61	4′45″	1,000
Bird #514						
12-14	3	3′25″	2,494	2	4′40″	1,000
12-15	32	3′	2,231	16	5′	1,000
12-16	131	3′	2,304	75	4′20″	1,000
12-17	203	3′	2,202	142	5′	1,000
12-18	95	3′	2,124	122	5′	1,000

COMPARISON OF METHODS

Table I presents the data from a study of 5 birds infected with our 3H2 strain of *Plasmodium cathemerium*, each bird being represented by a smear obtained at 9:30 A.M. on the first five days of its infection. The counting by our method was done on the dates as shown during a period when these birds

were being employed as untreated controls in a routine study. The counting by the "standard" method was done at a later time, thus increasing the validity of the comparison, since it is highly unlikely that the same fields were selected for study in both instances. It will be seen that in using our method the count on the first day always required more than three minutes, since some time was employed in finding the first organism; but the counts of the succeeding days required only the arbitrary three-minute period. In the "standard" counts the time required varied from four minutes and twenty seconds to five minutes,

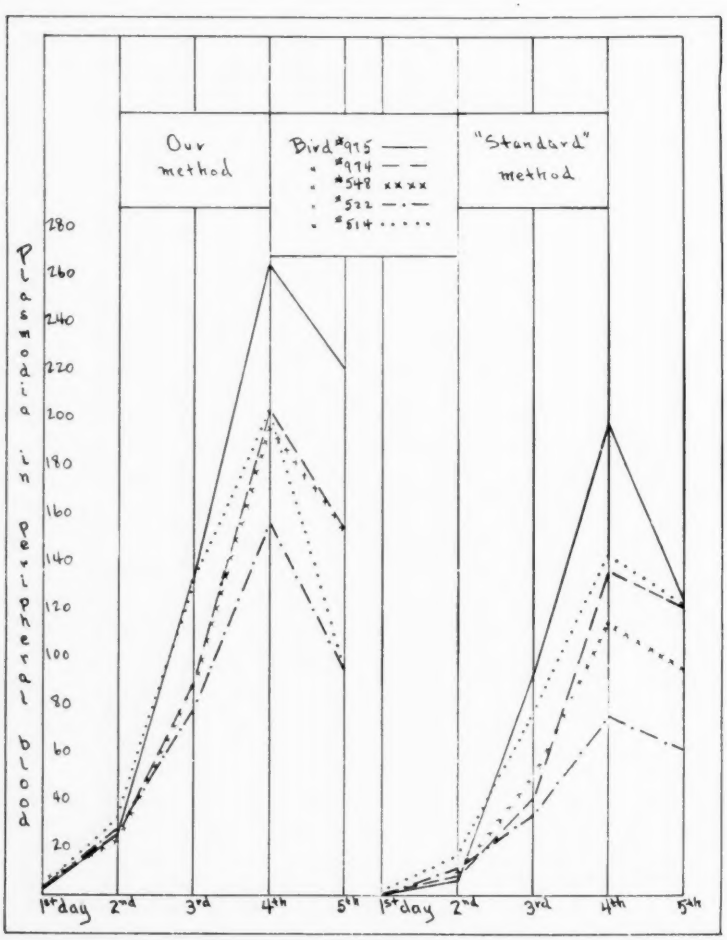

Chart I.—Course of the *P. cathemerium 3H2* infections in five canaries during the first five days as counted by the "standard" method and by our new method.

the average being four minutes and thirty-seven seconds. In the "standard" method 1,000 erythrocytes are examined; in our method more than twice this number are examined on all days after the first day; the extremes are 2,124 and 2,351, the average being 2,252. On the first day in our method we examine an average of 2,810 cells, or nearly three times as many as are examined in the "standard" method.

Chart I presents the counts in graphic form, the number of organisms being plotted as ordinates and the days of the infection as abscissae. The resemblance of the curves obtained by the two methods of counting seems to us in the main rather striking.

DISCUSSION OF OUR METHOD

We like our method for the following reasons: (1) No separate counts of erythrocytes and plasmodia are necessary; one merely gives the slide a hurried preliminary examination to select a suitable portion for study (a maneuver which precedes the use of all methods) and then begins examining erythrocytes and, almost incidentally, counting the plasmodia encountered. (2) There is astonishing uniformity in the number of erythrocytes examined, as shown in Table I, for the number of fields seen during the three minutes depends entirely upon the amount of time which must be devoted to the respective fields, more time being required of course to look separately at each erythrocyte in a crowded than in a scattered field. One quickly learns to omit fields in which only a few erythrocytes are present for this would obviously lead to error since the eye spends too much time in traversing unoccupied spaces, but this is easily accomplished by clicking off the stop watch and then clicking it on again when a more satisfactory area has been found. Then, too, no assumption of uniformity need be made with our method, since within reasonable limits satisfactory counts can be made, though the erythrocyte population of various fields differs quite widely. Each erythrocyte is looked at for the same length of time; that is the essence of the method. (3) By examining erythrocytes a full three minutes before declaring a smear negative we are reasonably sure it is really negative, for we have found upon innumerable occasions that a second study of three minutes and even a third practically never turns up a plasmodium. Furthermore, this three-minute study often enables us to find a plasmodium which would have been missed had we merely searched 1,000 erythrocytes; there is one such instance in Bird 548, as shown in Table I and Chart I. In our *P. cathemerium 3H2* infections the number of organisms increases sixfold to sevenfold in average instances between the first and second days; the necessity for detecting organisms on the very first day is therefore imperative if one wishes to begin treatment at the earliest possible moment.

As with all methods this has some disadvantages, but the only serious one of which we are as yet aware is that it can be employed only if one uses a stop watch having a mechanism to exclude periods of interruption and record only the actual time spent in examining erythrocytes rather than the total elapsed time. Nearly every laboratory, however, possesses one or more such watches. Of course a period of training is required to make one adept in the use of the method, but this is true of all precise procedures. We have found that for two workers to be able to check each other's counts requires only that they work together for a little while to adjust the amount of time actually spent in looking at each erythrocyte and also the speed of shifting from field to field.

SUMMARY

We have described a new method of counting plasmodia in avian malaria infections. Neither arbitrary numbers of erythrocytes nor plasmodia are

counted, but instead the slide is examined for an arbitrary period of three minutes, during which each erythrocyte is looked at individually and the plasmodial count is made rather incidentally to this examination of the erythrocytes. Despite the fact that one studies fields which vary considerably in density in using this method, the total number of erythrocytes examined varies only slightly from slide to slide. This method requires less time for its employment than the "standard" method, but enables one to examine more than twice as many erythrocytes. We believe the new method also permits earlier detection of patency than does the "standard" method.

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STREPTOCOCCAL HEMOLYSIS IN VARIOUS BLOOD MEDIA*

EUGENE F. TRAUT, M.D., AND MARY S. JOHNSON, B.S., CHICAGO, ILL.

CULTURES are commonly classified as hemolytic or nonhemolytic on the basis of their appearance as surface colonies on blood agar plates or slants. This classification becomes of considerable importance in view of the selective action of sulfanilamide preparations in therapy.

Most investigators have demanded of hemolytic strains of streptococci not only the production of hemolysis on an unspecified kind of blood plate but also more exacting criteria, usually the hemolysis of definite suspensions of red blood cells of specified origin. Other workers have encountered strains whose hemolyzing character depended upon the kind of blood used. This variability in hemolysis is often a source of confusion in the exchange of cultures between laboratories using blood from different sources. On moving our laboratory from the John McCormick Institute for Infectious Diseases, where sheep's blood had been used, to Rush Medical College where we used human blood, we noted that some stock cultures consistently nonhemolytic had become hemolytic. We investigated the constancy of this important change in cultural characteristics. We also applied to our strains the tests for hemolysis advocated by Brown.[1]

Cummings[2] favors inoculating suspensions of red cells to determine hemolysis. He regards as hemolytic only those streptococci that uniformly lake human, guinea pig, and mouse blood. Those strains producing hemolysis of only certain bloods he calls pseudohemolytic. Neither hemolytic nor "pseudohemolytic" strains change chocolate agar. He found "pseudohemolytic" streptococci regularly less virulent than the hemolytic.

*From the Laboratories of the Presbyterian Hospital and Rush Medical College.
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According to Brown[1] the green-producing streptococci and pneumococci produced less hemolysis in human blood agar than in horse or rabbit blood agar. The amount of hemolysis produced by B streptococci was insignificantly less on human than on horse or rabbit blood agar. Brown recognizes hemolysis only if produced within poured agar plates or in suspensions of red cells.

To hemolyze is usually regarded as a constant, unvarying characteristic. Strains have rarely been reported as losing or acquiring this property when identical conditions are reproduced.

Brown, Irons, and Nadler[3] noted a decrease in hemolyzing ability after repeated subculture. Dana and Murray[4] describe two strains of streptococcus pyogenes as hemolyzing horse blood but as producing green and not causing hemolysis in human blood. They found another strain producing green surrounded by a hemolytic zone in sheep and rabbit blood but typically hemolyzing all other bloods. They conclude that "since there are so few variations and since these may be due to some outside influence such as immune bodies in the blood, it would seem safe to assume that the hemolytic property of streptococci is fixed and constant."

Callow[5] says "the fact that a number of strains of nonhemolytic streptococci from the blood of rheumatic and nonrheumatic patients become hemolytic after prolonged cultivation may indicate that the hemolytic function can be suppressed."

METHODS

All blood plates were made by mixing 10 c.c. of fresh defibrinated blood with 100 c.c. of agar that had been melted and cooled to the proper temperature. The plates were incubated for 24 hours before being inoculated. Cultures were streaked out until individual colonies could be identified. These colonies were studied microscopically and macroscopically after 24 and 48 hours of incubation at 37.5° C. They were then re-examined after 24 and 48 hours' refrigeration. Five-tenths c.c. of broth culture of the same strains grown 24 hours in peptone ascitic broth was added to 1 c.c. of a 5 per cent suspension of washed sheep erythrocytes in normal saline. At the same time, a similar suspension of human cells was inoculated with these same strains. The supernatant fluid of these suspension cultures was examined for hemolysis after one hour in the refrigerator. The inoculations of a given strain on human and sheep blood were made on the same day, and the effects of colony growth were noted at identical times. Other observers concurred in our observations of the bacterial growths.

Because dextrose may inhibit hemolysis, it was not used in any of our media.

Of the 17 strains of streptococci studied, 12 were isolated from the rectum according to a previously described method.[6] Two were found in throats, one in the urine, one in the blood, and one was grown from the skin in erysipelas. Two strains of hemolyzing colon bacilli were obtained from the rectums of two patients. One strain of hemolyzing colon bacilli was obtained from a specimen of urine.

RESULTS

One strain of streptococci (Bo) consistently hemolyzed human blood while producing no hemolysis in sheep blood. This strain produced green on sheep blood on one subculture, and 2 months later was indifferent on the same medium. It never produced green on human blood. Two strains of streptococci (Oss, Endo) hemolyzed sheep blood but not human blood. Six strains of streptococci (Gen, Wym, Hal, Mil, Mon, erysipelas) hemolyzed cultures of both bloods but hemolyzed sheep blood more. One strain (Wym^2) produced more hemolysis in human blood than in sheep blood. Nine strains, including the culture from scarlet fever and the 3 strains of hemolyzing colon bacilli, hemolyzed human and sheep blood typically and to the same extent.

SUMMARY

The ability or inability of several strains of streptococci to hemolyze depended partly or wholly upon the origin of the blood used to enrich the culture medium. Hemolyzing ability or lack of it remained constant at least four months for a given type of blood used in culturing. Besides an absolute difference between colonies hemolyzing one kind of blood and failing to hemolyze another, there were clear-cut quantitative differences between amounts of hemolysis produced in the two kinds of blood. The variety of blood that was to make up a medium to test hemolysis might be either sheep or human but was always the same for a given strain. Taken individually, there was a great regularity in the reaction of these strains as regards hemolysis in a given kind of blood. In the group of bacteria tested, human blood was as often hemolyzed as sheep's blood.

The hemolyzing or nonhemolyzing character of a given strain remained constant for a given type of blood regardless of the method used to test hemolysis.

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OBSERVATIONS BASED ON A LARGE SERIES OF COMPLEMENT TITRATIONS

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DURING the past four or five years the successful application of the Kolmer complement fixation test in routine testing of blood specimens has been due, we believe, to the use of high titer complement. In recent conversations with various serologists who represented many laboratories, however, the author was much surprised to hear the many complaints regarding complement titration.

In the Public Health Laboratories of the North Dakota State Department of Health little trouble has been experienced with complement per se, when used in the Kolmer complement fixation test. In view of the difficulties exercised in the complement titration, it was deemed worthwhile to point out the experience in North Dakota. Two points bear out the above statement: (1) the consistently low titer of complement as used in other serologic laboratories, and (2) the available records of high titer complement as used in the North Dakota Public Health Laboratories.

From experience most serologists will agree that for the efficient performance of complement fixation tests, good complement is a decided asset. The use of poor complement invariably gives a "sloppy" run, accompanied by slow clearing of the test tubes with the end result of a large number of doubtful readings which are eventually proved to be negative.

Many authors have pointed out the importance of diet upon the quality of complement; however, no attempt will be made here to cite any specific literature. A complete picture of the entire system used in North Dakota should suffice, and it is hoped some benefit may be derived therefrom.

CARE OF GUINEA PIGS

The guinea pigs used as a source of complement are full-grown male or nonpregnant female animals purchased on the open market. No particular breed is especially purchased. The pigs are purchased when small and grown to maturity in our laboratories. The animals are quartered inside the year around, with the temperature of the room varying from 60° to 80° F., depending upon outside conditions. They are kept in metal cages of a size two by four feet, averaging from 10 to 12 per cage. Males and females are kept separately. The cages are cleaned once a day and animals are fed each morning.

The diet consists chiefly of Purina Rabbit Chow Checkers (entire ration) manufactured by the Ralston Purina Company, St. Louis, Mo. The ingredients are ground oats, corn meal, wheat germ, soy bean oil meal, corn germ meal,

*Director, Division of Laboratories, North Dakota State Department of Health.
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alfalfa meal, wheat middlings (gray), molasses, riboflavin supplement, 1.5 per cent calcium carbonate (limestone), 0.5 per cent iodized salt. A portion of these checkers is kept in front of the animals at all times. During the winter the pigs are fed freshly cut carrots at least once a day and during the summer freshly cut grass is substituted for the carrots. Hay is placed in the cages twice a week and year around. Fresh water is put in each cage every morning and twice a day in extremely hot weather. With this diet our experience has been that the pigs gain weight steadily and always look "well."

BLEEDING OF ANIMALS

All feed is removed from guinea pigs at least eighteen hours before they are bled, although water is allowed in the cages. Usually from 6 to 10 pigs are bled at each period. Approximately 8 c.c. of blood is collected from each animal. The pigs are bled about once every six weeks.

The animals are lightly anesthetized with ether and bled from the heart with a 20 gauge needle and a 10 c.c. syringe. The blood from each animal is placed in a separate test tube and allowed to remain at room temperature for approximately one hour. The tubes are then placed in the refrigerator overnight.

The following morning the serum is poured from the clots and centrifuged for ten minutes at approximately 1,500 revolutions per minute. The complement is pooled and placed in tubes containing about six c.c., then placed in the freezing compartment of an electric refrigerator at a temperature of approximately –8° C.

TITRATION OF COMPLEMENT

Complement prepared as above gives a high usable titer for approximately three weeks. The hemolysin used is of a high titer (generally 1:8,000) and is purchased from a commercial house. The antigen used is purchased directly from Dr. John A. Kolmer in Philadelphia.

The amount of complement needed for the day's run is thawed by placing tube in a small amount of cold water about thirty minutes before titration is to be set up. A 1:30 dilution of complement is used in the titration. All saline used in the titration is kept in the refrigerator in a large flask; hence cold saline is used throughout.

In a series of ten test tubes the complement titration is set up as shown in Protocol I.

It will be noted that after the complement antigen and saline are added the tubes are placed in water bath at 37° C. for only thirty minutes; after the addition of hemolysin and corpuscles, the titration is read when the tubes have been in the 37° C. water bath for only twenty minutes. As in all complement titrations, the smallest amount of complement just giving complete sparkling hemolysis is the exact unit. The next higher tube is the full unit which contains 0.05 c.c. more complement.

Table I shows the results obtained on 612 complement titrations by closely adhering to the above procedure.

Examination of Table I indicates clearly that most of the complement titrations show a titer of 0.25 c.c. or over. Of the 612 titrations, 84.8 per cent

gave a titer which could be used in the complement fixation test of 1:50 or above. This means that only 15.2 per cent of all complement titrations gave such titer that complement was used below 1:50. Further study shows that 11.0 per cent of those below 1:50 were usable at a dilution of 1:43. That the titers obtained were high is indicated by the fact that in 14.4 per cent of the titrations, complement was used at a dilution of 1:75 or higher. The largest number of titrations (70.4 per cent) showed that complement could be used at a dilution of 1:50 and 1:60.

PROTOCOL I

TUBE	COMPLEMENT, C.C. (1:30)	ANTIGEN DOSE, C.C.	SALINE SOLUTION, C.C.	Water bath 37° C. for 30 min.	HEMOLYSIN, C.C. (2 UNITS)	CORPUSCLES, C.C. (2 PER CENT)	Water bath 37° C. for 20 min.
1	0.1	0.5	1.4		0.5	0.5	
2	0.15	0.5	1.4		0.5	0.5	
3	0.2	0.5	1.3		0.5	0.5	
4	0.25	0.5	1.3		0.5	0.5	
5	0.3	0.5	1.2		0.5	0.5	
6	0.35	0.5	1.2		0.5	0.5	
7	0.4	0.5	1.1		0.5	0.5	
8	0.45	0.5	1.1		0.5	0.5	
9	0.5	0.5	1.0		0.5	0.5	
10	None	None	2.5		None	0.5	

TABLE I

RESULTS OF 612 COMPLEMENT* TITRATIONS
JANUARY 1, 1939 TO DECEMBER 31, 1942

NUMBER OF TITRATIONS	EXACT UNIT C.C.	FULL UNIT C.C.	PER CENT OF TOTAL NUMBER	TWO FULL UNITS DILUTION TO USE
17	0.1	0.15	2.8	1:100
71	0.15	0.2	11.6	1:75
201	0.2	0.25	32.8	1:60
230	0.25	0.3	37.6	1:50
67	0.3	0.35	11.0	1:43
16	0.35	0.4	2.6	1:37
7	0.4	0.45	1.1	1:33
3	0.45	0.5	0.5	1:30
	0.5	0.55		1:27
612			100.0	

*Frozen guinea pig serum used in all titrations (1 day to 3 weeks old).

The complement giving a titer of 0.15 c.c. and 0.1 c.c. were those titrated generally within three days after being obtained from guinea pigs. The opposite was found to be true, in that those titers on the lower end of the scale were generally encountered when complement was from 17 to 21 days old.

CONCLUSIONS

1. The successful application of the Kolmer complement fixation for syphilis in North Dakota has been due to a large extent to the use of high titer complement.

2. The care of guinea pigs, especially diet, is essential to the production of high titer complement.

3. Complement kept in a frozen state has proved to be highly satisfactory when not over three weeks old.

4. Scrupulous care as to technique will result in good complement titrations.

THE DERMOFLUOROMETER*

An Instrument for Objective Measurement of the Fluorescence of the Skin and Organs and the Objective Determination of Circulation Time and Capillary Permeability

Kurt Lange, M.D., and S. E. Krewer, New York, N. Y.

SOME time ago Lange and Boyd[1] showed that fluorescein, injected intravenously as a 5 per cent solution and irradiated by long wave ultraviolet can be seen in the capillaries of the skin and mucous membranes. This permits the determination of circulation time to different parts of the body and the establishment of adequacy of blood supply to these structures.[2, 3] Fluorescein can be watched traveling with the blood stream, permeating the capillary wall, and staining the tissue cells.

This method can be used routinely as a simple procedure to locate exactly the site of a vascular occlusion[2] or to ascertain how much blood flow to an organ or section of skin is diminished by the difference in depth of staining per unit of time. Extensive studies made it obvious that an awareness of an area of diminished blood supply was extremely valuable; quantitative measurement of the amount of fluorescein present at a given moment in any district of the skin or mucous membrane added considerable objective information.

Fluorescence of this dye is excited by blue and violet visible light as well as by an ultraviolet light source. This makes it possible to employ an incandescent lamp provided certain precautions are taken. While the ultraviolet light source equipped with adequate filters is unsurpassed for demonstration and visualization of vascular lesions, incandescent blue light is very convenient for actual measurements since it is smaller and produces less heat.

When we adopted the incandescent lamp for exciting fluorescence, it became feasible to design a compact unit which combines the light source as well as the phototube in rigid relative positions to each other (Fig. 1). The exciting (primary) blue light impinges upon the body while the yellow-green fluorescent (secondary) light (i.e., light of longer wave length than the primary light), of the blood stream, evoked by the blue light, is registered by the phototube; it is necessary to prevent the primary light from reaching the phototube by reflection from the skin or organ surface. This is accomplished by a filter F_1 (Fig. 2), in the primary light beam and filter F_2 set in front of the phototube. Filter F_1 transmits blue light only and absorbs practically all light of a wave length *longer* than 5000 Angstrom units. Conversely, filter F_2

*From the Department of Medicine, New York Medical College, Flower and Fifth Avenue Hospitals and Metropolitan Hospital-Service (Research Unit).

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absorbs all light of a wave length *shorter* than 5000 Angstrom units and transmits mainly yellow-green light characteristic of the fluorescence of fluorescein. Since all these filters have a small undesirable leakage in the infrared, the phototube selected for this work is entirely insensitive to infrared radiation.

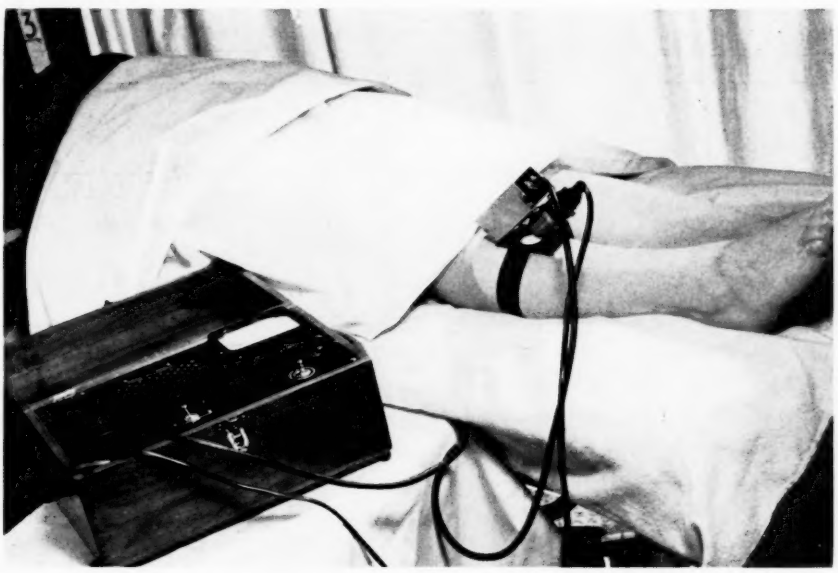

Fig. 1.—The dermofluorometer. The search unit comprising the light source and the phototube is attached to the leg of a patient.

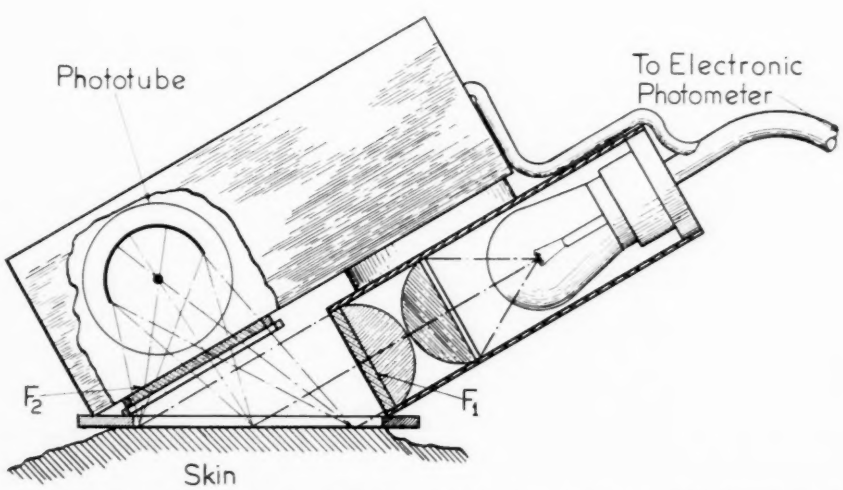

Fig. 2.—Schematic drawing of the search unit. F_1 blue filter in front of light source, F_2 yellow filter in front of phototube.

Because of these provisions, the light registered by the phototube before injection is negligible and is taken care of by a "background reading," which gives the actual degree of deflection of the instrument before the injection of fluorescein. The deflection following the injection of fluorescein therefore rep-

resents the effect of this injection quantitatively. The current output of the phototube is amplified approximately 3×10^5 in the electronic photometer.*

Table I shows the deflections of the instrument when standard solutions of fluorescein are tested; it proves that the device deflects proportionately to the concentration of fluorescein employed.

TABLE I

DILUTION OF FLUORESCEIN	DEFLECTION OF INSTRUMENT
1: 500,000	43.0
1: 1,000,000	22.0
1: 5,000,000	4.5
1:10,000,000	2.4

The instrument is used as follows: In a darkened room, several test spots of the body to be examined are touched with the instrument and the "background" deflection is noted. The instrument is then attached with a usual rubber strap to the spot at which the circulation time is to be observed. Ten cubic centimeters of a 5 per cent fluorescein solution are injected intravenously, and the time elapsing between the beginning of the injection and the initial deflection of the instrument is measured with a stop watch. This constitutes the circulation time to this area. A mouthpiece made from lucite, a translucent material, can be used to determine the circulation to the lips or gums if this is preferred to visual observation. Subsequently, all test spots are touched with the instrument, and the deflections of the photometer are read. From the results thus obtained, "background" readings are subtracted in order to obtain absolute fluorescein values. Thus a curve is obtained for each area of the body surface which indicates how much blood and, with it, fluorescein is reaching this district per time unit.

To prove that these deflections are really dependent upon blood supply, a blood pressure cuff was put around one thigh of a patient and inflated to a pressure between systolic and diastolic level. Subsequently comparative readings were taken on the normal and on the compressed leg of the patient, and the values were noted. Such a test is shown in Fig. 3. It indicates clearly that the values in the leg with partial occlusion are considerably lower than those in the normal leg.

To standardize the values found with different instruments, the following basis was adopted: one skin unit is the deflection caused by an alkaline fluorescein solution of 1:30,000,000 in a cuvette of 5 millimeters depth, the glass of which shows no absorption for the exciting light.

The first part of the curve thus obtained depends primarily upon the amount of blood reaching a given area per unit of time and upon capillary permeability. It will be shown in another paper that changes in plasma proteins within physiologic limits do not influence the amount of fluorescein available for diffusion into the tissue fluids. The last part of the curve is strongly influenced by the excretory function of the kidney.

Fig. 4 shows a typical normal curve recorded at the leg, and the curve of a patient with arteriosclerosis of the extremities, and one from a patient suf-

*The complete instrument was manufactured for us according to our specifications by the Photovolt Corporation, New York, N. Y

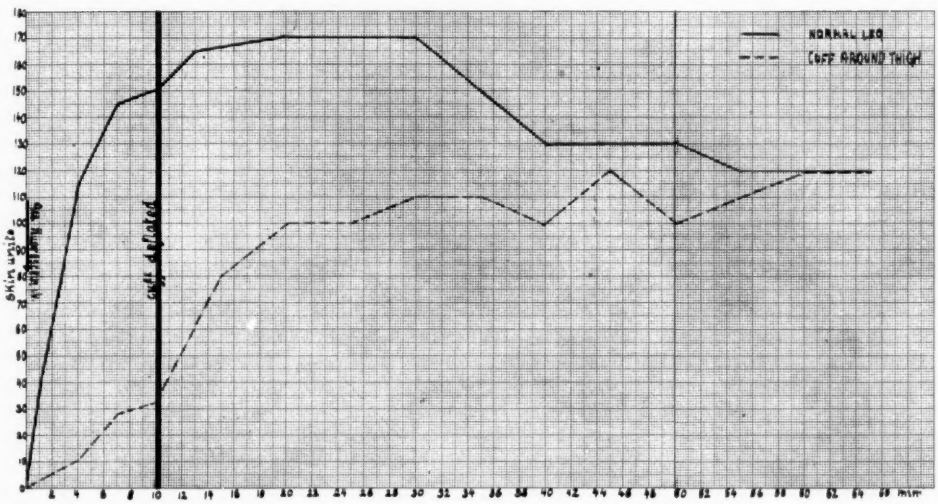

Fig. 3.—Dermofluorometer readings in a patient who had a blood pressure cuff inflated to a pressure between systolic and diastolic blood pressure around one thigh. 10 c.c. fluorescein were injected intravenously. Ten minutes after injection the pressure in the cuff was released.

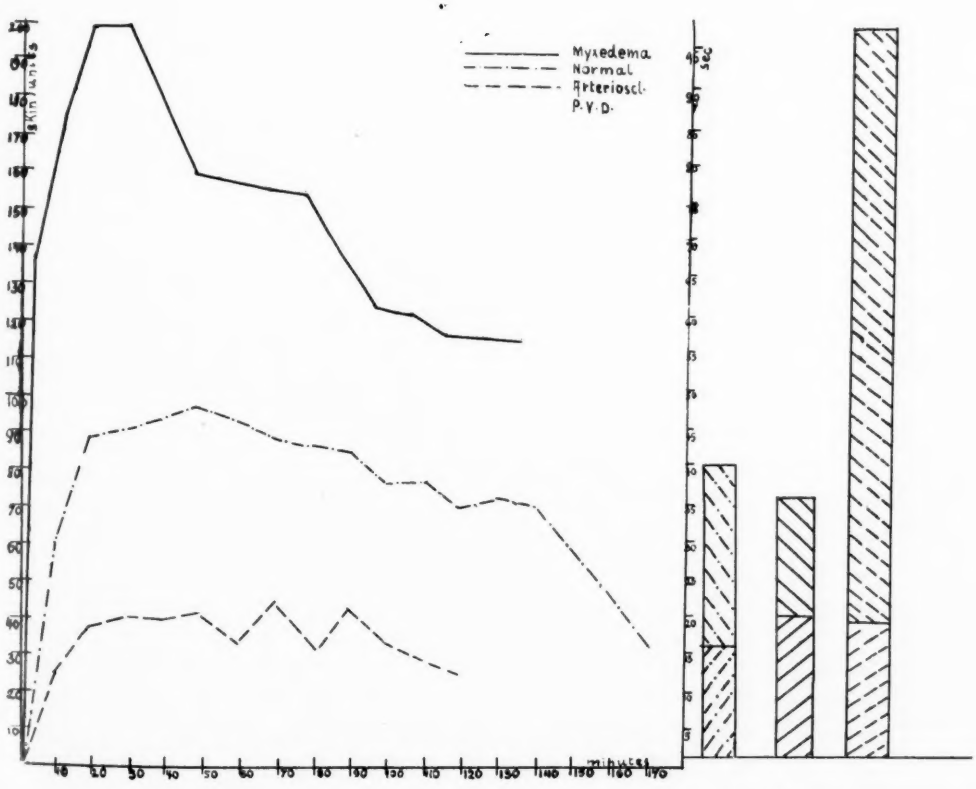

Fig. 4.—Dermofluorometer curves of three patients, one of whom is suffering from generalized arteriosclerosis, one from myxedema, and one, a normal person. The blocks give the circulation time in seconds from the arm to the lips and to the right leg respectively for each case.

fering from myxedema. In myxedema, the capillary permeability is especially high, and this may be the explanation for the peculiar skin condition and the tendency to form transudates, especially in the pericardium. By applying the instrument to different parts of the extremity of an individual with peripheral vascular disease, very often the exact place at which the artery narrows decidedly can be foretold. At the corresponding skin level, skin fluorescence suddenly decreases. At the same time the circulation time to a limb with a generalized arteriosclerosis is usually markedly prolonged. In a normal person the arm to leg circulation time should not exceed twice the arm to lip time. Formerly this could be seen with long wave ultraviolet, but now it can be measured exactly with the dermofluorometer.

SUMMARY

The instrument described permits quantitative measurements of the fluorescence values obtained when patients with vascular disorders are tested with fluorescein. A skin unit of fluorescein is defined. The exact location of a vascular stenosis can be determined with this device. Capillary permeability can also be determined.

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RAPID DIAGNOSIS OF MALARIA BY THE USE OF A WRATTEN LIGHT FILTER

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CONTRAST filters are necessary in photography of colored or stained objects but can also be utilized in clinical microscopy. The visual contrast afforded can shorten the period of search required in a number of microscopic examinations before a negative report is issued.

A diagnosis of malaria after a short search for the parasite is not the rule. Many oil immersion fields must be examined before one intracellular organism is seen. When the young malarial forms, the "signet-ring" merozoites, are present, the closest study is required. In an early case or in a recurrence, the relative infrequency of infested red cells may cause the issuance of a false negative report. The paleness of the blue cytoplasm, often thinned out to little more than a line, allows an understandable oversight in running through hundreds of red cells. This is particularly true if no mature and larger forms are encountered, a not unusual condition.

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The thick drop method of crowding the red cells into the oil immersion field does not often disclose the intracellular location of the parasite. It is difficult to increase the staining intensity of the malarial organism by the routine hematologic stains.

A suitable light filter will produce the maximum contrast between the parasite and the red cells without of course affecting the stain for routine study. Blood stains are essentially a combination of methylene blue and eosin. It was found that of the various Wratten light filters* the E light red filter (series 23A) has a spectral transmission range parallel to that of eosin.[1] Moreover, this filter transmits a higher percentage of incident light in the red part of the spectrum than does eosin. Consequently the red cells actually appear lighter. The absorption of all blue (and green) light by the E filter renders the blue-stained elements distinctly darker. This contrasts sharply with the pale shadow or "ghost" rendition of the red cells. In a specified examination period 3 to 4 times as many parasites can be located as with normal lighting.

The simplest and least expensive form of the E filter is the dye-stained gelatin film supplied in one- or two-inch squares obtainable at photographic supply stores. A two-inch square can be cut up into a number of filters between cardboard mounts. These can easily be fitted to cover either the substage condenser or the ocular, and likewise can be instantly removed to study the located parasite by straight lighting.

Where analagous problems in microscopic studies exist, the enhanced contrast of light filters may similarly be utilized.

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*Wratten light filters, Eastman Kodak Co., Rochester, N. Y.

MACROSCOPIC BLOOD TYPING*

A Modification of the Method of Thalhimer and Myron

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THE macroscopic method of blood typing, when compared with the standard microscopic method, offers advantages of economy in time and equipment and ease of performance and reading. It makes typing afield, such as outside of hospitals and under war conditions, simple and practical. If it can be shown to equal or exceed the microscopic method in accuracy, it would seem to be the method of choice. A gross method of typing has been described by Thalhimer and Myron[1] for use with the globulin fractions of pooled sera, the idea apparently being that the serum concentration thus attained would furnish agglutinating titers adequate for such a test. The method consists essentially in putting a large drop of A and B serum concentrate side by side on a card; placing a small drop of the undiluted blood to be tested at the side of each drop of serum; mixing the two drops with the adjacent blood; and, after a minute's rotation, observing the presence or absence of agglutination.

We used these concentrated sera and the method with the same satisfactory results indicated by Thalhimer and Myron, but while doing so and employing unpooled and unconcentrated sera for comparison, it was noted that single sera not infrequently gave equally good or superior agglutination. This suggested to us the possible value of an appraisal of unmodified single sera for the purpose. Accordingly, about 1,500 random sera were tested in various ways pertaining to the application of this method, controlling all results by the microscopic method. Sundry aspects of the results are submitted below.

Undiluted Versus Diluted Blood.—Undiluted blood taken directly from the finger necessitates an immediate performance and reading of the test but, on the other hand, requires little equipment and time and may be more desirable at the bedside and outside of hospitals. Blood diluted with an anticoagulant may be tested at one's convenience as to time and place and permits repetition of the test when desired. Our preference for diluted blood is based on the results of typing over 200 donors with both undiluted and diluted blood macroscopically and diluted blood microscopically. Here it was found that typing with undiluted blood was more open to error in reading results than with diluted blood, as the greater concentration in the former does not permit as clear a view of the developing agglutination. Likewise, it was noted that typing with anemic undiluted blood gave a clearer picture than normal concentrated blood for the same reason.

A blood dilution which gave a more readable agglutination than the use of pure blood and yet was not so diluted as to weaken the picture was one part

*From the Clinical Laboratories, Hahnemann Hospital. Technical assistance was given by K. E. Simmons and M. C. Krause.
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of blood to two parts of diluent. This dilution, while very satisfactory for macroscopic tests, is too low for microscopic work, which requires quite dilute suspensions.

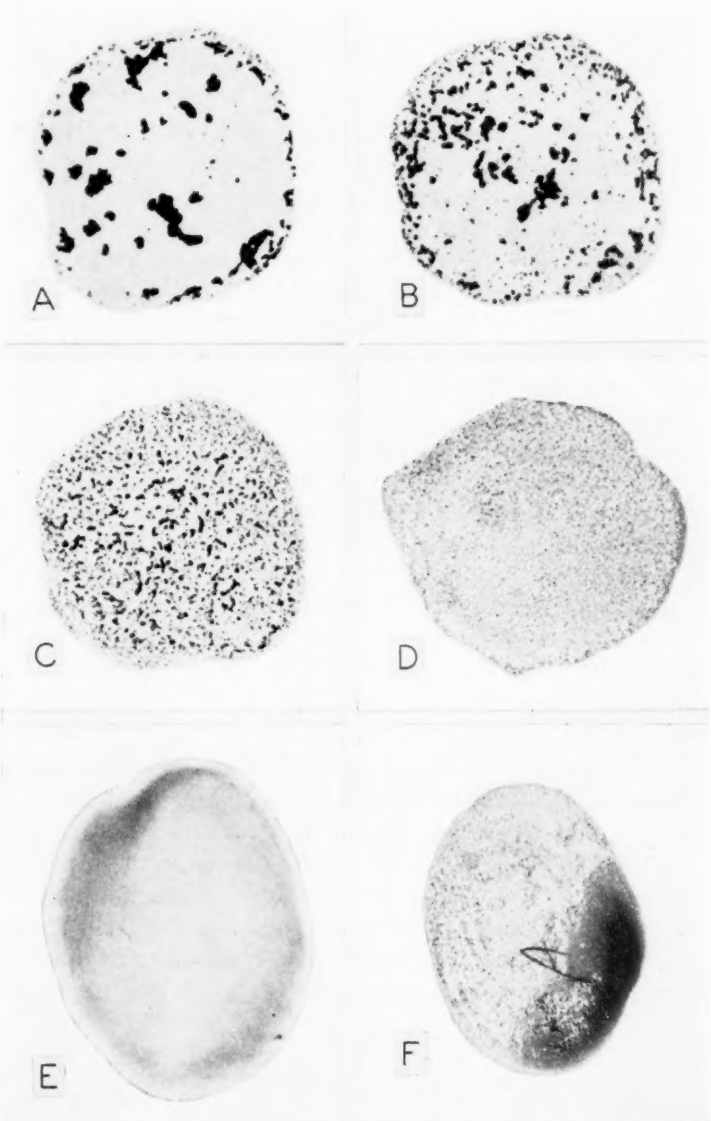

Fig. 1.—*A*, *B*, *C*, and *D* photographs show various degrees of macroscopic agglutination of red calls; *E* illustrates absence of agglutination; *F* photograph shows false clumping as seen occasionally in dried mounts.

The Blood Diluent.—Sachs,[2] in a study of the role of salt concentration in blood grouping tests, found that hemagglutination with unheated and heated serum was hastened and intensified in a high salt concentration, and he recommended that the cells for blood grouping be suspended in a 2 or 3 per cent sodium chloride solution. He stated that suspending the corpuscles in this stronger sodium chloride solution is a safe way of avoiding false negative reac-

tions such as may occur on cross matching. He also found that although the speed and strength of agglutinations are at first much increased with high salt concentration, the end titer may be lower.

On this basis, gross blood typing tests were done with a suspension of one part of blood in two parts of a 1 per cent solution of sodium citrate in 2 per cent sodium chloride. Comparison of the results with this solution with those attained with a 0.9 per cent saline solution confirmed the claims of Sachs. It was found that gross agglutination appeared uniformly more promptly in the 2 per cent salt suspension and that the advantage, while not great, was of sufficient value to warrant the use of the stronger solution.

Evaluation of Sera for Typing.—As a first preliminary test, an effort was made to type 100 unselected samples of blood serum by the gross method, and this was successful in all but 2 sera in which the titer was too low to give conclusive clumping. In a second preliminary test, an attempt was made to type 100 red cell suspensions using as typing sera A and B samples of the random sera of the first experiment. In doing this, sera of average or less than average agglutinating strength were purposely employed instead of the stronger sera. The entire 100 blood suspensions were thus typed correctly.

Having shown that blood may be readily typed by the macroscopic method using either known sera or known red cell suspensions, the problem was studied more exactly in a series of tests of donors' sera by noting carefully the time of gross agglutination and the size and character of the clumps and comparing these with the agglutinating titer of the respective serum. There were thus tested and titrated 100 A and 100 B sera. It was found that the isohemagglutinin titer and the agglutinative ability as measured macroscopically did not always agree quantitatively, and that in occasional individual cases, there was a marked discrepancy,* an experience similar to that of Thalhimer and Myron.[1] On the whole, however, as these authors point out in their work, there was a general trend toward correlation. A serum with a titer above 1/80 was usually adequate for gross typing. But for the purposes of the test, it was found better to rely on the gross results as seen in the rapidity and readability of the reaction than the titer. The rapid production of coarse clumps is the macroscopic evidence of maximum agglutination. Sera producing beginning agglutination in ten seconds or less and complete agglutination within

*In the titration tests, five dilutions of serum in normal saline were made as follows: 1:20, 1:40, 1:80, 1:160 and 1:320. To 1 c.c. quantities of such dilutions in small test tubes was added 1 drop of a 1:3 fresh suspension of appropriate red cells. This mixture was allowed to stand an hour at room temperature, observed for agglutination, and then placed in the refrigerator overnight to be given a final reading in the morning. The first list below illustrates average comparative readings of titer and macroscopic clumping. The second illustrates selected examples of discrepancies in such readings: it is only fair to say that these are rare.

TYPE	TITER	GROSS CLUMPING	TYPE	TITER	GROSS CLUMPING
A	1:40	++	A	1:40	++++
A	1:320	++++	B	1:40	+++
A	1:80	++	B	1:160	++
A	1:160	+++	A	1:20	++
A	1:40	+	B	1:80	++
A	1:20	+			
B	1:80	+++			
B	1:40	++			
B	1:320	++++			
B	1:20	++			

one minute were graded ++++. Sera producing beginning agglutination in ten to fifteen seconds and complete agglutination in sixty to seventy seconds were graded +++. It is important to note that complete clumping may be present within these limits in the form of fine or medium-sized masses, though larger clumps may form later on standing. Sera grading 3 or 4 plus were serviceable for macroscopic typing. Thus measured, about 20 per cent of the A sera and 40 per cent of the B sera met these standards, so that one may anticipate that 1 out of 5 A sera and 2 out of 5 B sera will be adequate for macroscopic tests. It is especially important to use strong B sera on account of the subgroups of A bloods, and this is not difficult as strong B sera are quite common.

In the same way, fifty O sera were grossly tested and titrated against suspensions of A and B cells. Again it was found that the macroscopic clumping reaction afforded better indications of the agglutinative ability of the serum than titration. Usually O sera are stronger in anti-A agglutinins than anti-B agglutinins. In 50 sera, the anti-A were stronger than the anti-B agglutinins in 34 instances; in 15 instances, the anti-A and anti-B agglutinins were approximately equal in strength; and in one case, anti-B agglutinin was stronger. Of fifty O sera, 34 gave a 3 or 4 plus clumping reaction against A cells, and 12 gave a 3 plus clumping against B cells. As measured by macroscopic clumping, about 2 out of ten O sera may be expected to give a 3 plus or greater agglutination with both A and B suspensions. Such sera are adequate for control tests.

Cross typing of blood as a preliminary to blood transfusion involves the use of sera of unknown and perhaps low agglutinin values whether the tests are done by the macroscopic or microscopic method. In a series of 100 routine cross typings of donor and donee, in which both methods were applied, the gross method agreed with the microscopic test throughout. In our routine cross typing, however, we still employ both the gross and microscopic methods, as we wish by continued observation to be sure of the accuracy of the gross tests before dropping the standard microscopic one. Our further experience suggests this will be possible.

Reading of Results.—If the blood typing is done in the usual way, i.e., using A and B ++++ or +++ type sera against the donor's or recipient's red cell suspensions, the gross clumping is so prompt and self-evident in the great majority of instances that the type may be read macroscopically within a minute (Fig. 1 *A, B, C, D,* and *E*). If, on the other hand, an unknown serum is to be typed with A and B red cell suspensions, the rapidity and degree of agglutination are dependent on the unknown agglutinin titer, and may, in a serum poor in agglutinins, exhibit slow action and poor clumping, though it is nearly always possible to be certain of the type. Such results are quite exceptional, and doubtful cases, when controlled by typing through the blood suspensions, usually prove to be the type originally suspected.

With the usual large- and medium-sized clumps, the reading is perfectly simple. Infrequently, when the clumps are fine and formation slow, the reading is delayed and sometimes difficult. Here and in other instances, the additional use of a high titer O serum may be helpful. For example, if there is no

clumping with A or B serum and definite clumping with O serum, the results are erroneous. Again, when there is doubtful or unsatisfactory agglutination with A or B serum, the absence of clumping with O serum makes it fairly certain the type is O. If, after the use of O, A, and B sera, there is still doubt, the test should be reversed by trying the gross agglutinative effects of the serum of the blood to be tested against known emulsions of A and B cells. Under such circumstances, it should be kept in mind that if the serum to be typed is an O, it will usually clump A cells very promptly before there is any visible clumping in the B cells. It is therefore essential not to cease rotation of the card when A clumping appears, thus mistaking it for B serum, but to prolong it to be sure that the B cells also will not clump.

Final readings for type should be done while the mount is freshly wet. Card paper of the thickness recommended warps quickly under the influence of the blood-serum mixture, and correct reading is more difficult if the test is put aside to be read later. We do not feel it is worth while to preserve the dried individual tests as part of the clinical record, as the appearance changes somewhat in the dried specimen and in certain cases may give a false impression of type. In drying or dried blood-serum mixtures, a fine granulation may develop and be mistaken for true clumping by the inexperienced. (Fig. 1 *F*.) Negative bloods, that is, those like type O which show no clumping with A or B serum, are of necessity rotated longer in watching for possible agglutination; but this should not be continued too long, for example, four or five minutes, as a fine false clumping may be apparent.

One should not get the impression from the foregoing details, which are given largely as precautionary information, that macroscopic typing is a matter of hazards and difficulties. On the contrary, we found macroscopic typing quite as reliable as and more easily performed than the microscopic method. In the case of a type O patient showing weak irregular isoagglutinins which clumped not only her own cells but all other O types, the aberration was picked up quite as well by the gross as the microscopic method. And in the case of a sailor, typed by the Navy and wearing an O tag, the macroscopic method detected the error and showed he was an A type.

EQUIPMENT

A, B, and O ++++ or +++ typing sera in drop bottles. Keep, in intervals, in refrigerator.

White card index paper or No. 2 Bristol board cut in strips 10 × 4 cm.

Blood diluent, 1 per cent sodium citrate in 2 per cent sodium chloride.

Capillary pipettes and nipples. Small test tubes (7.5 × 1.3 cm.). Toothpicks.

Typing sera are easily obtained by taking an extra test tube of blood when bleeding donors. When ten or more of these sera are on hand, appraise them by the macroscopic method against A and B blood suspensions. Two or more out of ten will have a gross clumping value of +++ or ++++ and are serviceable for macroscopic tests.

METHOD

Place 8 drops of diluent, measured with a capillary pipette, in the small test tube, and add to it, collected with the same pipette, 4 drops of blood from

the patient's or donor's finger. This makes a 1 to 3 dilution which will keep; it is of the proper density and sufficient in quantity for repeated tests. Immediately or at one's convenience, place a large drop of each test serum, at least 7 mm. in diameter, on a labeled card strip, and beside it, a small drop of the diluted blood, one-fourth to one-third of the size of the serum drop. Both serum and blood drops will assume and retain an almost globular form on card paper. If two types of sera are used, the order from left to right should always be A and B; if three are used, the order should be A, B, and O. Mix each diluted blood and the respective serum with the flat end of a separate toothpick, spreading the mixture to a circle about 3 cm. in diameter. Avoid small circles. At once rock the card in various directions for a minute; let stand for twenty to thirty seconds; rerock the card for ten to twenty seconds. Read results while freshly wet. In rare instances, when clumping is slow or indistinct in one or both mixtures, further rotation may be necessary, but this should not be protracted beyond two or three minutes, as a fine false clumping may lead to incorrect readings. Dried mixtures not infrequently show this fine false granulation.

SUMMARY

In a study of the macroscopic method of blood typing described by Thalhimer and Myron, the following modifications are recommended: the use of diluted blood; a diluent of high salt concentration; the use of single, unconcentrated typing sera of high gross agglutinative ability. The reasons for these changes are discussed.

In our experience with about 1,500 controlled tests, the macroscopic blood method of blood typing here described equaled the microscopic method in accuracy and was superior to it in rapidity, simplicity, and economy. It has now been used as the routine method in our laboratories for four months with equally satisfactory results.

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AN INHIBITION PHENOMENON IN PRECIPITATION TESTS FOR THE SERODIAGNOSIS OF SYPHILIS*

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THE purpose of this communication is to point out an inhibition phenomenon that may be a source of error in the precipitation test for the serodiagnosis of syphilis. The colloidal state of some sera is such that, although reaction between antigen and reagin apparently takes place, the formation of visible aggregates is inhibited. A serum of high activity may thus appear to be of only slight activity or to be nonreactive. The properties of the sera that give rise to this phenomenon are not understood; but the technique of precipitation tests should be such that sera of this character may be recognized and their reactivity interpreted correctly.

The inhibition phenomenon has been observed occasionally in an oversensitive procedure,[1a, 2] and there is reason to suspect that it may occur also in other tests. Although inhibition of precipitation may occur simultaneously with a prozone reaction,[3, 4] early recognized as a source of error, the two phenomena appear to be distinct and should not be confused. The properties of serum that inhibit precipitation do not disappear on dilution with physiologic saline solution. It has been found, however, that centrifugation† of the tests of such sera will cause the formation of large flaky aggregates. Similar treatment of typically negative or weakly reacting test mixtures does not cause precipitates of this character.

In Table I are presented the results of precipitation tests performed on four successive specimens of blood serum from a patient undergoing malarial therapy for syphilis of the central nervous system. The sera were tested undiluted and in a series of dilutions in physiologic saline solution with the antigen adjusted for oversensitive preliminary tests. With the first three specimens, reactions originally of insignificant degree became marked on centrifugation for five minutes at about 2500 revolutions per minute. The fourth specimen gave typical results. The attending physician knew of nothing in the patient's history to account for the change in serum properties.‡

These same specimens were examined with two other antigens for precipitating activity. When tested in a presumptive procedure,[5] the first three specimens failed to react, while the fourth specimen reacted as follows: undiluted, 2+; diluted: 1:5, 4+; 1:10, 4+; 1:20, 4+; 1:30, 4+; 1:40, 4+; 1:50, 4+; 1:60, 3+; 1:80, 2+; 1:100, +; and 1:150, –. They were also examined, for pur-

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†Centrifugation is a routine step in some precipitation procedures.

‡The sera from thirty-one other patients similarly treated did not show this peculiar property.

poses of comparison, with a carefully adjusted mixture of relatively pure lecithin and cardiolipin* sensitized with cholesterol. In this instance typical results were obtained by the regular procedure without centrifugation (Table I). Thus, although the colloidal condition of a serum is such as to inhibit the formation of a precipitate, this irregular behavior is also influenced by the antigen.

TABLE I

READINGS OF PRECIPITATION REACTIONS WITH SUCCESSIVE SPECIMENS OF BLOOD SERUM FROM ONE PATIENT

SERUM DILUTED WITH PHYSIOLOGIC SALINE	SPECIMEN NO. 167353			SPECIMEN NO. 170476			SPECIMEN NO. 172247			SPECIMEN NO. 175800		
	TEST WITH OVERSENSITIVE ANTIGEN		TEST WITH CARDIOLIPIN ANTIGEN	TEST WITH OVERSENSITIVE ANTIGEN		TEST WITH CARDIOLIPIN ANTIGEN	TEST WITH OVERSENSITIVE ANTIGEN		TEST WITH CARDIOLIPIN ANTIGEN	TEST WITH OVERSENSITIVE ANTIGEN		TEST WITH CARDIOLIPIN ANTIGEN
	REGULAR	CENTRIFUGED	REGULAR	REGULAR	CENTRIFUGED	REGULAR	REGULAR	CENTRIFUGED	REGULAR	REGULAR	CENTRIFUGED	REGULAR
Undiluted	−	2+	2+	−	2+	3+	−	2+	3+	+	Not	4+
1:2	−	2+	4+	−	2+	4+	+	3+	4+	3+	done	4+
1:4	±	3+	4+	±	3+	4+	2+	3+	4+	4+		3+
1:8	+	3+	3+	+	2+	3+	2+	3+	3+	4+		2+
1:16	+	2+	2+	±	2+	2+	+	3+	2+	4+		+
1:32	+	2+	+	−	2+	+	−	2+	−	3+		−
1:64	−	+	−	−	−	−	−	+		2+		−
1:128	−	−		−	−		−	−		+		
1:256	−	−					−	−		−		

Specimens were from a patient who had syphilis of the central nervous system and was undergoing malarial therapy; all had titers greater than 10 in the quantitative complement fixation test.[1b]

TABLE II

REPORTS OF PRECIPITATION TESTS WITH TWO SPECIMENS FROM THE WASHINGTON SEROLOGY CONFERENCE

SPECIMEN NO.	OVERSENSITIVE PROCEDURE	TEST A	TEST B	TEST C	TEST D
830	3+, +, ±, −* Positive Atypical	Positive	Negative	Doubtful	Negative
1090	±, ±, +, −** Doubtful Atypical	Negative	Positive	Positive	Positive

*The last three tubes containing 0.1 c.c. of undiluted serum and serum diluted 1:5 and 1:25 respectively with 0.02 c.c. of antigen, when centrifuged, read 4+, 4+, 3+.

**The first tube containing 0.05 c.c. of serum and 0.08 c.c. of antigen and the second and third tubes containing 0.1 c.c. of undiluted serum and of serum diluted 1:5 with 0.02 c.c. of antigen each, when centrifuged, read 4+, 4+, 4+.

These two specimens had titers greater than 10 in the quantitative complement fixation test.[1b]

Some of the discrepant results obtained in different test procedures may be due to this phenomenon. Table II records a few of the results obtained with two specimens, No. 830 and No. 1090, in the Washington Serology Conference.[2] Both specimens were from patients with diagnoses of syphilis of over four years' duration. Based on the results of the oversensitive procedure as performed routinely, the specimens were reported as "atypical positive" and "atypical

*The lecithin and cardiolipin were kindly supplied by Dr. Mary C. Pangborn.[6]

doubtful," respectively. Subsequently the tests were centrifuged, and evidence of marked reactions was obtained. The conflicting reports suggest that doubtful and negative results may be misinterpreted owing to failure to recognize the inhibition phenomenon.

The problem is one that should be considered for individual precipitation procedures; methods should be developed where necessary by which specimens that require special treatment may be detected. The recognition of the phenomenon in a given instance would require the use of more than one reaction mixture to indicate atypical findings.

CONCLUSIONS

The precipitation test for the serodiagnosis of syphilis is evidently subject to a phenomenon of inhibition in which the reaction between serum and antigen becomes manifest only under certain conditions. This inhibition appears to be distinct from the prozone phenomenon although the two may occur together. The phenomenon is of sufficient importance to require further investigation and careful consideration in the standardization of the technical procedure of precipitation to avoid the possibility of a false interpretation of the results.

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CHEMICAL

MODIFICATIONS IN METHODS FOR THE PRECIPITATION AND ASSAY OF INCREASED AMOUNTS OF PITUITARY GONADOTROPIC SUBSTANCES IN THE URINE*

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THE demonstration of those pituitary gonadotropic substances in the urine which are follicle-stimulating has depended upon their precipitation and subsequent assay in a chosen test animal. Zondek (1930) precipitated urine with alcohol and used the ovarian response of immature rats as the end point of assay. Hamburger, in 1933, and Albright, Halsted, and Clony, in 1935, used the alcohol precipitation method of Zondek, but observed the ovaries of infantile mice. Thomsen and Pedersen-Bjergaard (1936) used tannic acid and studied uterine enlargement together with ovarian weights in rats, and Callow and Callow (1937) modified their tannic acid method of precipitation. Levin and Tyndale (1936) also used tannic acid and were the first to use the weight of the infantile mouse uterus as the end point of assay. Catchpole, Greulich, and Sollenberger (1938) reported a method in which tungstic acid was the precipitating agent and in which mouse uterine weights were recorded. Heller and Heller (1939) used alcohol precipitation together with ovarian and uterine weights in immature rats as the test objects. In the experience of this laboratory all of the above procedures have been tried with the exception of the method in which tungstic acid was used, and no one procedure has proved entirely satisfactory. A method has therefore been developed which gives consistent and satisfactory results: alcohol was chosen as the precipitating agent; the volume used was increased, and enlargement of the uteri of infantile mice was the criterion of a positive test. Besides its use in the study of the menopause and in the sorting out of the various types of hypogonadism in males and females, it has led to the elucidation of two clinical conditions reported by Albright, Smith, and Fraser (1942) and Kleinfelter, Reifenstein, and Albright (1942).

CHOICE OF PRECIPITATING AGENT

Both alcohol and tannic acid as precipitating agents received extensive trial in this laboratory. The former has given excellent results; the latter as here

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employed* was not consistently satisfactory (see Table I). At the suggestion of Fevold (1939), eight volumes of alcohol instead of four were employed, and the data showing the efficacy of alcohol as compared with tannic acid are given in Table I. Since certain patients after the menopause have so much pituitary gonadotropic substances in the urine that an assay can be made by injecting such urine without precipitation into animals, yields obtained by this procedure (so-called "direct method") were compared with those obtained by precipitation with alcohol or tannic acid followed by redilution.

Study of the table reveals that positive assays were obtained more frequently with the use of alcohol than with the use of tannic acid. Thus, of the ten cases studied, six showed better yields with alcohol than with tannic acid (Cases 1, 2, 3, 4, 9, and 10); in four cases (5, 6, 7, and 8) the yields were the same; in no instance was the yield with tannic acid better. The reason for the complete failure of the tannic acid method in certain instances (e.g., Cases 1 and 3) remains obscure. Since good results were obtained in some instances, it is conceivable that there is some error in technique in the method as used.

CHOICE OF METHOD OF BIOLOGIC ASSAY

In the selection of the method of biologic assay one may choose the rat or the mouse as the test animal and the ovary or the uterus as the significant organ of response. Hamburger (1933) pointed out that the mouse ovary was a more sensitive test object for pituitary gonadotropic substances than the rat ovary. Albright, et al. (1935) confirmed this. Levin and Tyndale (1937) reported data in the use of the uterine weights in immature mice as the criterion of a positive test but used tannic acid as the precipitating agent. This laboratory has followed the procedure of Levin and Tyndale in the use of the mouse uterine weight but has departed from their described procedure in that (a) alcohol was the precipitating agent, (b) different standards of normal uterine weights were established (videinfra), and (c) a different method of evaluating a positive test was used. Furthermore, it is strongly felt in this laboratory that quantitations of the active substances present should depend on response to various dilutions rather than on the degree of response of the test organ.

CRITERIA FOR NORMAL AND FOR ENLARGED MOUSE UTERI

To establish values for normal uterine weights in uninjected 21-day-old albino female mice weighing between 5 and 10 grams, 98 animals were autopsied and body and uterine weights recorded (Table II). The coefficient of correlation was 0.26 ± 0.06. This coefficient is more than four times its probable

*The method used was a modification of Callow and Callow (1937): A solution of analytical reagent tannic acid is freshly prepared just before use; 3 c.c. of a 10 per cent solution of tannic acid per 100 c.c. of urine are added to a concentrated first-morning specimen which has been filtered through Reeve Angel filter paper, No. 202, and pH adjusted to 4.5-5 before the addition of the tannic acid. The mixture is allowed to stand in the cold room 16 to 24 hours and the precipitate collected into 50 c.c. conical tubes by centrifuging. The precipitate is washed with 5 c.c. of acetone per 100 c.c. of urine volume four times and is placed in a suction desiccator overnight. The dry powder may be stored indefinitely without loss of potency; 3 c.c. of borax buffer (50 c.c. of 0.2 M boric acid (61.8 Gm./L = 1.0 M) in 0.2 M potassium chloride (74.6 Gm./L = 1.0 M) is mixed with 29.95 c.c. of 0.2 N sodium hydroxide and diluted to 200 c.c.) is added to the amount of dry powder obtained from 100 c.c. of urine, and 5 c.c. of distilled water is added. The mixture is stirred thoroughly, and 9 drops of phenol red added to the mixture are used as an indicator. Adjustment to pH of 8-9 is made by the addition of a 5 per cent solution of sodium carbonate, and the entire mixture is allowed to stand at room temperature for no less than one hour: 9 more drops of phenol red are added and the pH now adjusted to 7-7.5, using 2 per cent acetic acid. Distilled water is then added to make a final volume of 25 c.c. This gives a solution, each 2.5 c.c. of which is equal to 10 mouse units per 100 c.c. of the original urine.

TABLE I

RESULTS OF THREE METHODS OF ASSAY FOR PITUITARY GONADOTROPIC SUBSTANCES IN URINE

SOURCE OF MATERIAL	METHOD OF ASSAY					
	DIRECT		TANNIC ACID		ALCOHOL	
	M.U./100 C.C.	RESULT	M.U./100 C.C.	RESULT	M.U./100 C.C.	RESULT
Case 1. C. W. MGH 231528	40	+	20	Negative	20	+
	67	+	50	Negative	50	+
	100	+	70	Negative	70	Negative
Case 2. G. B. MGH 231481	40	Negative	10	+	10	+
	67	Negative	30	Negative	30	+
	100	Negative				
Case 3. E. R. MGH 32958	40	Negative	10	Negative	10	+
	67	Negative	30	Negative	30	+
	100	Negative	50	Negative	50	Negative
Case 4. A. S. MGH 17995	67	+	10	+	10	+
	100	+	30	+	30	+
	150	Negative	50	Negative	50	+
Case 5. A. S. MGH 17995	40	+	10	+	10	+
	67	+	30	+	30	+
	100	+	50	+	50	+
	150	Negative	70	+	70	+
			100	+	100	+
Case 6. A. M. MGH	40	+	30	+	30	+
	67	+	50	+	50	+
	100	+	70	Negative	70	Negative
	150	Negative	100	Negative	100	Negative
Case 7. H. K. MGH	40	+	10	+	10	+
	67	+	30	Negative	30	Negative
	100	+	50	Negative	50	Negative
			70	Negative	70	Negative
Case 8. A. S. MGH 17995	40	+	10	+	10	+
	67	+	30	+	30	+
	100	+	50	Negative	50	Negative
Case 9. E. R. MGH 32958	40	+	10	+	10	+
	67	+	30	Negative	30	+
	100	Negative	50	Negative	50	+
Case 10. Pooled spec., patient E. R. (MGH 32958) and patient A. S. (MGH 17995)	40	+	10	+	10	+
	67	+	30	Negative	30	+
	100	Negative	50	Negative	50	+

TABLE II

DATA SHOWING BODY AND UTERINE WEIGHTS IN 21-DAY-OLD FEMALE ALBINO MICE WEIGHING BETWEEN 5 AND 10 GRAMS

WT. OF ANIMAL GM.	UTERINE WEIGHTS MG.					TOTAL NO. ANIMALS	AV. UTERINE WEIGHT MG.
	2.0-2.9	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9		
5.0-5.9	4	5	2			11	3.27
6.0-6.9	6	10	2	2		20	3.45
7.0-7.9	4	11	6	3		22	4.13
8.0-8.9	3	16	7	4	1	31	3.93
9.0-9.9	3	5	4	2		14	3.80

error and is therefore significant, although the significance is of slight degree. If the data in the group of animals weighing less than 7 grams are excluded, the correlation between the weight of the animal and the uterine weight is insignificant, so that if animals within the weight range of 7 to 10 grams are used, it is unnecessary to apply any correction factor to the uterine weights of in-

jected animals. To establish the fact that the injection of fluid alone had no effect on uterine weights, four normal mice received three times the volume of fluid used in the test in the form of normal saline. No uterine enlargement was noted.

In the series of normal control animals the average uterine weight (Table II) was 3.7 ± 0.94 mg. The uterine weight can fluctuate by chance alone within the limits of twice the standard deviation, or from 1.7 mg. to 5.5 mg. Even though the variation just given would cover 95 per cent of possible normal weights in those animals whose body weights lie between 5 and 10 grams, it was felt that a higher value, e.g., 6 mg., for the upper limit of normal would add significance to a positive result in a routine laboratory procedure. Therefore, a uterine weight of 6 mg. has been regarded as of beginning significance. The exact method of determining a positive test is presented.

PROCEDURE*

Precipitation.—Sixty c.c. of a concentrated first morning specimen of urine is filtered into a 1-litre Erlenmeyer flask. No specimen is accepted unless the specific gravity is at least 1.010. The pH is adjusted to 4.5-5, using nitrazene paper as the indicator and 75 per cent acetic acid as the acidifying agent. Eight volumes of cold alcohol which has stood at a temperature of not more than 24° C. are added. The mixture is rotated thoroughly and allowed to stand at this temperature for twelve to forty-eight hours. The active principle, which is water-soluble, settles to the bottom as a precipitate. The flask may remain at the reduced temperature for as long as a week without altering the results of the subsequent assay. The supernatant fluid is removed by suction (or by decanting if it has stood for the longer period of time), leaving a small amount behind in order to rinse out the precipitate into a 50 c.c. Pyrex conical centrifuge tube. All of the precipitate is collected into the conical centrifuge tube by use of a rubber policeman and repeated rinsings of the Erlenmeyer flask with supernatant fluid. The precipitate remains in the centrifuge tube throughout the subsequent steps of the procedure. After complete collection, it is washed twice with absolute alcohol, once with anhydrous ether, and placed in a desiccator overnight. There result a dry powder which can be stored indefinitely without loss of potency if kept dry. The active principle is prepared for assay by dissolving the powder in 15 c.c. of distilled water and adjusting the pH to 4.5-5 (as tested with nitrazene paper) using 75 per cent acetic acid. The water is allowed to stand in contact with the powder four to eight hours, and the mixture is stirred well at intervals no less than four times. If an insoluble residue remains, the clear supernatant fluid is used for injection. There is no loss of potency if this solution stands for several days, but loss of potency can occur, however, if the solution is allowed to stand as long as fifteen days at a temperature of 24° C. or less.

Whether complete precipitation is obtained even with alcohol is, of course, a debatable point. Comparison of the assays in which alcohol was used with the assays in which the direct method was used (Table I) show some discrepancy. One explanation may be incomplete precipitation; another may be that there

*The procedure here given has been slightly modified in the hands of Kleinfelter, Albright, and Griswold (1943) in that calculations are made in mouse units per twenty-four hours rather than mouse units per 100 c.c.

are substances in the unprecipitated urine which have a direct effect on the mouse uterus causing enlargement and which are removed in the various steps of the precipitating procedure. The discrepancy needs further elucidation.

Assay.—The assay is carried out on three 21-day-old mice, weighing between 7 and 10 grams. Each animal receives a total of 2.5 c.c. of the test solution prepared according to the procedure just described. Each mouse is injected as follows: 0.5 c.c. morning and afternoon of day 1; 0.5 c.c. morning and afternoon of day 2; 0.5 c.c. morning of day 3. Autopsy is performed the morning of day 4. Macroscopic enlargement of the uteri is considered a positive test. In doubtful cases the uteri are carefully dissected, pressed between filter paper to remove fluid, and weighed. The test is considered positive when the weight of the uterus is greater than 6 mg.

If only two of the three animals have uteri weighing between 6 and 7 mg., the test is not positive; but, if all three animals have uteri weighing between 6 and 7 mg., the test is considered positive. If one of three animals has a uterine weight of more than 7 mg. and the other two animals have uteri weighing less than 6 mg., the result of the assay is considered doubtful and is repeated not later than the next day. A conclusive result is usually obtained. When, according to this technique, three animals each receive 2.5 c.c. of the test solution and show no uterine enlargement, there is less than 1 mouse unit of pituitary gonadotropic substances present in 10 c.c. of the first morning specimen. When the mouse uterus is enlarged in animals which have received 2.5 c.c. of the test solution, the urine is known to contain a minimum of 1 m.u./10 c.c., or 10 m.u./100 c.c. In order to determine whether the gonadotropic content of the test solution is higher than 10 m.u./100 c.c., aliquots of this 15 c.c. of test solution are diluted from 2 to 9 times.* The assay is made with 2.5 c.c. of the diluted aliquots in the same manner as that described for the assay of the test solution.

DISCUSSION

Since the qualitative presence of pituitary gonadotropic substances can be demonstrated in instances where their concentration is high by injecting the urine directly into the test animals without preliminary precipitation, the point may well be raised whether testing for various levels is necessary. The injection of unprecipitated urine into the test animal gives a positive result only if the level is as high as 40 m.u./100 c.c. By alcohol precipitation the same volume of test solution injected is equivalent to 10 c.c. of the unprecipitated urine and, therefore, levels as low as 10 m.u./100 c.c. may be detected. For still lower values a modification of the Heller and Heller method is now used in this laboratory (Kleinfelter, Albright, and Griswold, 1943).

Using the method of concentration and assay just described, there was less than 10 m.u./100 c.c. in the urine of normal women throughout the menstrual

*In the use of three test animals a convenient table for making dilutions follows:

DESIRED ASSAY, M.U./100 C.C.	VOL. OF TEST SOLUTION NEEDED—C.C.	VOL. WATER TO BE ADDED—C.C.
30	3	6
50	2	8
70	2	12
80	2	14
100	1	9

cycle with the exception of one or two days at or near the time of ovulation. In menopausal patients any first morning specimen of urine was positive for a minimum of 10 m.u./100 c.c., and values as high as 100 to 150 m.u./100 c.c. were recorded in a few instances.

While enlargement of the uterus was the criterion of a positive test, it may be of interest that the ovarian response at the end of the 72-hour test period consisted of follicle enlargement only and corpora lutea were never seen. In addition, the vagina of test animals was patent in instances in which the level of the pituitary gonadotropic substance was of the order of 30 m.u./100 c.c. and over.

In the practical consideration of the test described, the greatest difficulty may be in obtaining animals which meet the specifications of the test. The procedure has merit in that the precipitate may be stored until such time as animals are available, and since the mortality rate of injected test animals is low, a minimum number of mice is needed.

SUMMARY AND CONCLUSIONS

1. In the method for the assay of increased amounts of pituitary gonadotropic substances in the urine the precipitation of those substances with alcohol is superior to precipitation with tannic acid.

2. Eight volumes of alcohol is probably better than four.

3. As a test animal, the mouse is more sensitive than the rat, and as a test organ, the uterus is preferable to the ovary.

4. The method used in this laboratory is presented.

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DOUBLE DISTILLATION IN ONE AND THE SAME FLASK*

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THE principle of the double distillation in one flask was described by us in 1934, and was put into practice by the construction of an apparatus for the preparation of double distilled water.† The described apparatus had the disadvantage of working only half automatically, as the water supply during the process of the distillation had to be regulated continually. These circumstances have induced us to construct the apparatus, reproduced in Fig. 1,

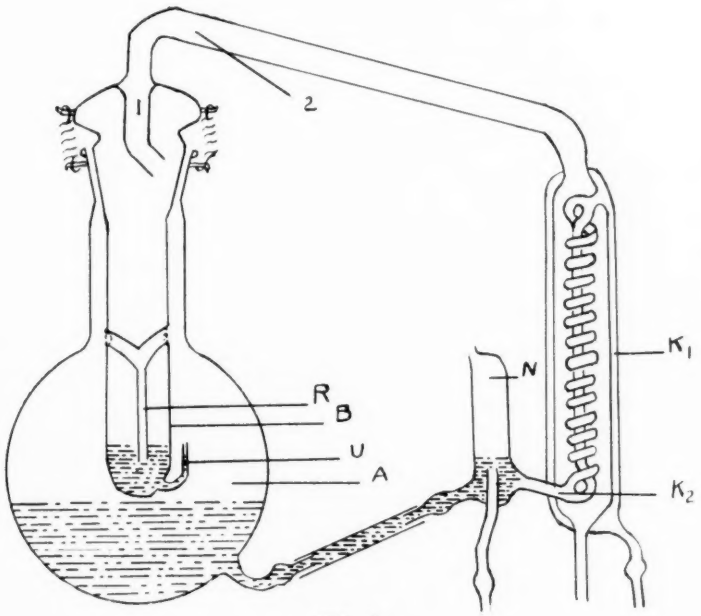

Fig. 1.

which guarantees a fully automatic double distillation. The apparatus consists of two distilling vessels (A and B), fitting into each other; A is heated by gas or electricity, B by steam created by A. The outer flask (A) is connected to a constant level arrangement (N), which allows a continuous water supply, thus replacing the water, evaporated in A. When the outer flask is heated, the steam gets into the inner distilling vessel (B) through Tube R, and from there via 1 and 2 into condenser K. By condensation in the condenser, part of the water runs down as singly distilled water. Another part, after condensation in Glass tube 1, runs back into vessel B and is condensed there, whereby the lower part of B up to the height of the drainage tube (U) is constantly filled

*From the Biochemical Laboratory of the Beilinson Hospital, Petah Tiqva, Palestine.
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†Rappaport, F.; Mikrochem. **15**: 302, 1934.

with water. The constant heating by the steam, coming in from R, and the heat in flask A effect a renewed distillation. The drainage tube (U) acts in a way as a valve, as the condensed water which does not reach a second distillation flows off, thereby obtaining a constant pressure and regular boiling. The inner vessel (B) is connected by way of ground joint and elastic wire spirals with A, and is directly melted to the double condenser, K_1 and K_2.

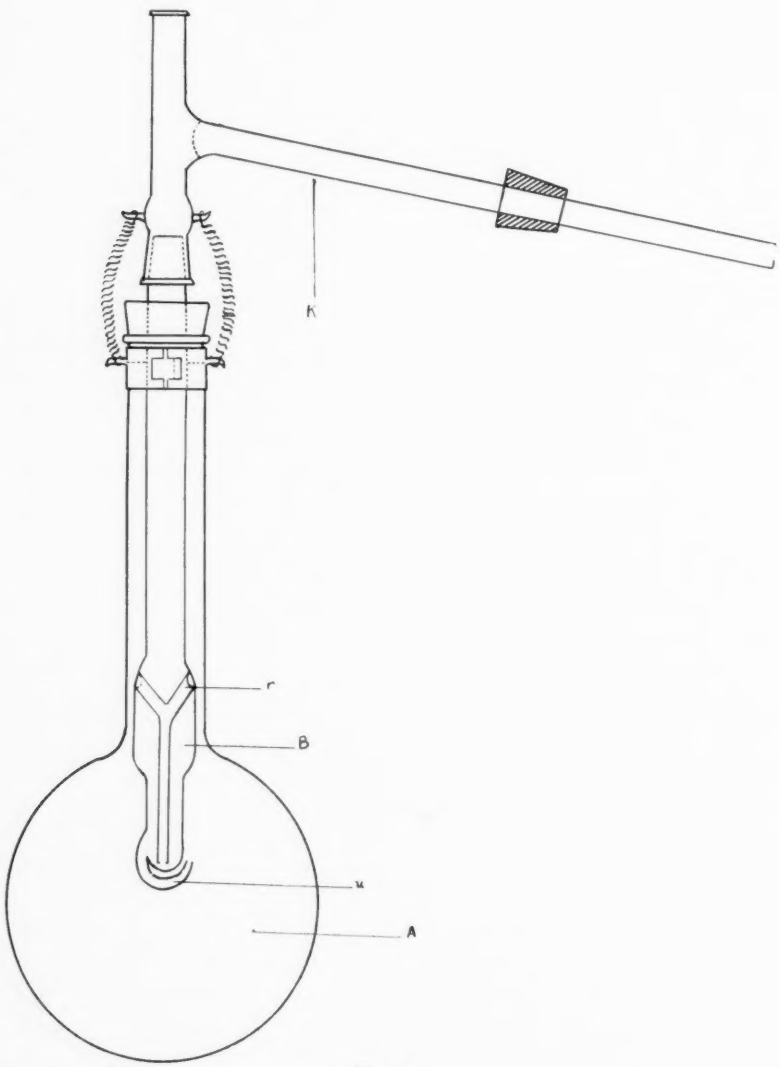

Fig. 2.

Thereby the apparatus becomes very handy and can be fixed on a stand. This apparatus supplies, provided appropriate dimensions are taken, approximately 1500 c.c. per hour. The water gained this way has a conductivity of $K = 1.5 .10—6$.

The principle of the one-sided double distillation can also be used for the rectification of different organic solvents with the aid of the apparatus,

reproduced in Fig. 2. The solvent is distilled from the outer round flask A into the inner vessel (B). Through continual heating by the steam coming through the Y-shaped tube (r), and also through steam present in bulb A, a second distillation into the inner vessel is effected. The water vapor gets into the distilling column (K) which is supplied with a thermometer and connected to a condenser. The column is connected by ground joint to vessel B which, according to requirements, can be fitted into flasks of different sizes by means of suitable stoppers. At the lower end of tube B a drainage tube (u) enables the condensates to run off during the distillation, thus effecting constant pressure in both vessels. The same principle of inserting two bulbs into one another

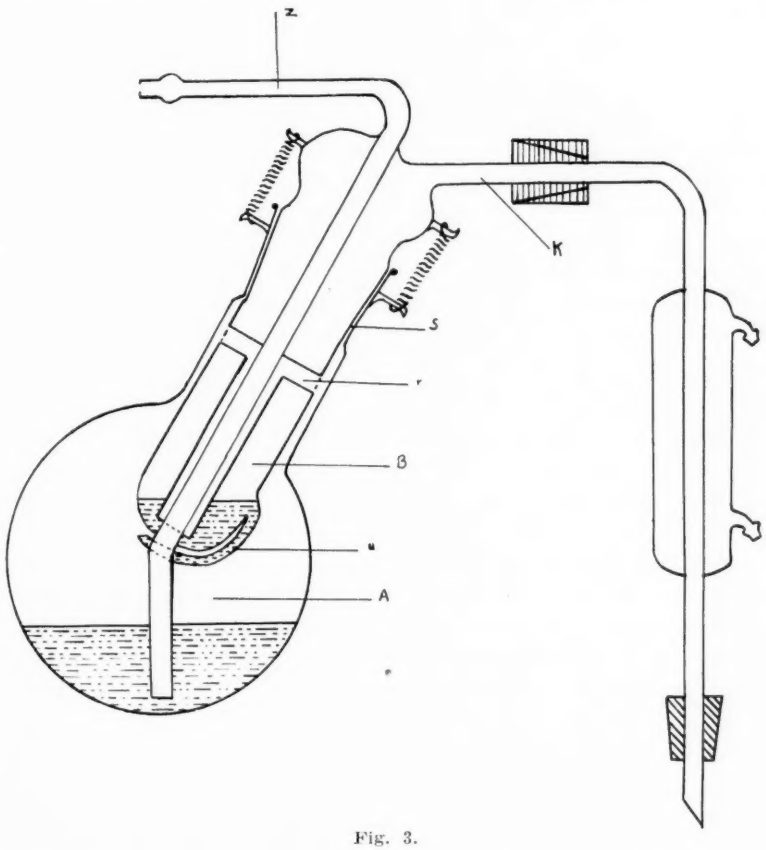

Fig. 3.

can also be profitably applied to steam distillation. (See Fig. 3.) Through tube z steam is led into the liquid that is immiscible with water in flask A. From there the steam reaches B over tube r. The condensing steam collects again on the bottom of the inner flask which communicates through drainage tube U with the outer flask and thereby stands under constant pressure. The oil-loaded steam, further produced in B, leaves through lead k into the condenser. This apparatus too may be furnished with stoppers instead of ground joints, and can thereby be connected with flasks of different sizes.

MICRODETERMINATION OF ACETONE AND DIACETIC ACID IN BLOOD*

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THE following method, which is based on the method of M. Ljungdahl,† allows the determination of acetone bodies accurately and quickly in 0.2 c.c. blood or serum by means of a simple and easily handled apparatus. The distillation apparatus (see Fig. 1) consists of three parts:

(1) The distilling flask (A), which is a 50 c.c. Erlenmeyer flask connected to the distilling headpiece by a ground joint.

(2) The distilling headpiece. When in use the connection is secured by a pair of elastic springs (*f*). The headpiece consists of a glass tube, the ends of which are bent downwards at an obtuse angle. The shorter side (a) is widened at its end to a semisphere (h) and is ground on to the distilling flask. The second and longer side (b) serves as a dropping tube and is surrounded by an external condenser tube for condensing the distillate. If there is no running water available, it is sufficient to fill the condenser with cold water once for each determination. The washing of the apparatus is done through the side tube (C), which is attached to the middle part of the distilling top piece. This tube (C) is extended to a capillary (D), running parallel to the axis. During the distillation, the tube is closed by a cork stopper. The glass rod, which is sealed on to the top of the semispheric ground joint and which ends closely above the water seal of the distilling flask, prevents boiling over.

3. The receiving vessel (B), a 50 c.c. Erlenmeyer flask, which is connected to the apparatus by a double clamp (S). One clamp holds the vessel; the second one is fixed to the condenser and can be moved so that the end of the distilling apparatus, according to requirement, is immersed into the received liquid or ends freely above it. To facilitate work during the distillation a hinge is fixed on the clamp carrier, thereby putting the flask in an oblique position and making it possible to shake it backwards and forwards as well.

REAGENTS

1. Diluted phosphoric acid solution. Two to three drops concentrated H_3PO_4 to 100 c.c. distilled water.

2. Iodine solution. 0.75 c.c. 1:10 N-Iodine solution and 5 c.c. 20 per cent sodium hydroxide are filled up to 50 c.c. with distilled water. (Prepared daily.)

3. Potassium iodide in crystals.

4. Concentrated hydrochloric acid diluted in equal parts with water in a dropping bottle.

5. 1:1000 n-sodium thiosulfate solution.

6. 0.25 per cent starch solution.

*From The Biochemical Laboratory of the Beilinson Hospital, Petah Tiqva, Palestine. Received for publication, April 8, 1943.

†Eine Mikromethode zur Bestimmung des Total-Acetons im Blute Biochem, Ztschr. 96: 345, 1919.

PROCEDURE

Before beginning the determination, the distilling apparatus is rinsed with distilled water through the side tube (C) and the opening is closed quickly with the stopper, so that a small column of liquid in the capillary (D) closes the latter against the inside of the distilling headpiece. The ground neck part of the flask (A) is moistened with a bit of water. By means of the elastic springs the flask is fixed on the semisphere of the distilling headpiece. On the other side of the apparatus a flask (B) is clamped. Then water is heated in A and distilled into B until cleanness of the apparatus is fully assured. Now the analysis is started with the determination of the "blank value."

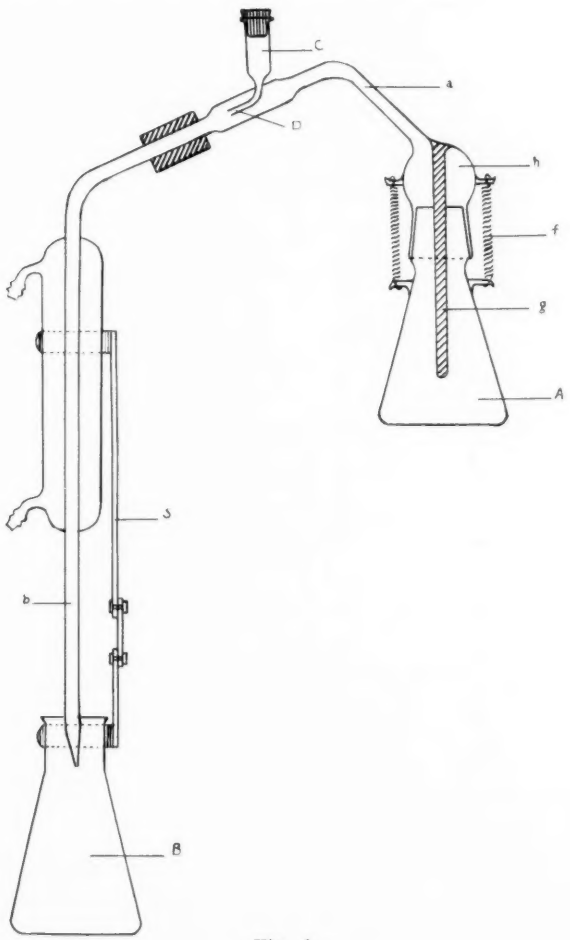

Fig. 1.

As receiving flask, flask B containing exactly 2 c.c. of the iodine solution (2) is fixed to the apparatus so that the distilling tube is immersed in the fluid. On the other side a distilling flask (A) containing 4 c.c. phosphoric acid is fixed in the manner explained above, and is heated by a small flame. The phosphoric acid is to be measured in a small, narrow-measuring cylinder in order to avoid mistakes arising from traces of alcohol, ether, or acetone left in the pipettes. When the contents of the flask have boiled for three minutes, flask

B is pushed down, so that the distilling tube is no longer immersed and goes on boiling for only another minute. Eventually distilled water is rinsed through the opening (C).

Some crystals of potassium iodide, hydrochloric acid, and a few drops of starch solution are put into flask B. It is shaken and then titrated with 1:1000 n-sodium thiosulfate solution. The value thus found is the "blank value." It must be equal to the value found when adding potassium iodide, hydrochloric acid, and starch solution to 2 c.c. of iodine solution in the same way in a receiving flask and when titration is carried out without any distillation. When this is not the case, the distillation must be repeated. Once the "blank value" is found, the actual determinations can be carried out, one after the other. This is done by adding, using the precautions necessary in exact analytic determinations, 0.2 c.c. of the blood or serum to be examined to the measured amount of phosphoric acid in flask A, which are attached to the apparatus in succession. Distillation is carried out just the same as described for the determination of the "blank value." Titration of the iodine solution in flask B, carried out subsequently, gives the full values.

CALCULATION

"Blank value" minus sample value multipled by 4.83 gives the acetone content in mg. per 100 c.c.

Example:

Blank Value	2.72
Sample Value	1.34
Difference	1.38

$1.38 \times 4.83 = 6.6654$ mg. per 100 c.c. acetone.

The figures given in the accompanying tables prove the utility of the method.

1. Amount of c.c. n/1000 thiosulfate solution required with diluted acetone solution.

Without Distillation	With Distillation
0.45	0.44
0.90	0.91
1.34	1.33
1.79	1.77

2. Blood and serum to which measured amounts of acetone were added always gave values that corresponded to the amount of acetone found in the blood plus that added to it.

Example:

Blood	0.78 c.c. n/1000 of thiosulfate solution
Acetone Solution	1.34 c.c. n/1000 of thiosulfate solution
Calculated Amount	2.12 c.c. n/1000 of thiosulfate solution
Blood Plus Acetone Solution (Value Found)	2.10 c.c. n/1000 of thiosulfate solution

SUMMARY

A method is described which allows the determination of the content of acetone in 0.2 c.c. blood or serum with great accuracy and a simple apparatus.

This work was begun in Vienna and completed in Palestine.

BOOK NOTICES

Chemotherapy of Gonococcic Infections*

TREATMENT of gonococcic infections with the sulfonamide drugs is presented from the standpoint of a streamlined program of practical value. Choice of drugs, dosage, and toxicity are fully discussed. Special mention is made of the infectious carrier and the sulfonamide resistant case. Various methods of treatment such as fever therapy combined with a sulfonamide and vaccine combined with a sulfonamide are discussed, as are treatments of specific complications.

Of particular value to the busy practitioner is the omission of discussions of history, bacteriology, pathology, and endoscopic examination and treatment.

The problem of diagnosis and determination of cure in both male and female are stressed. At the end, certain investigative problems are briefly presented, and case outlines are given to illustrate points in diagnosis, treatment, and complications.

The book has a high practical value to the practitioner as well as to the specialist, in its adequate, concise presentation.

Blood Groups and Transfusion†

THE third edition of Dr. Wiener's book *Blood Groups and Transfusion* has much new material, necessitated by the many advances in whole blood and plasma transfusion, as developed in the last few years. The presentation is very comprehensive. It includes discussion of blood groups and technique of grouping, selection of donors, indications for transfusions, methods of transfusion of whole blood, plasma, and serum, reactions and complications, and the organization and use of blood banks. There is a chapter on the history of transfusion, and sections are devoted to the heredity of blood groups, the newly recognized Rh factor, group-specific substances in organs and body fluids other than the blood, medicolegal application of blood tests, and technique for the identification of blood stains. There is a very interesting chapter on anthropologic studies of blood groups and on evolution of the human blood groups.

This volume should have a wide field of usefulness.

The Physiological Basis of Medical Practice‡

SINCE its first printing in January, 1937, Best and Taylor's *Physiological Basis of Medical Practice* has had three American editions, one Spanish, one Portuguese, and has had a total of twelve printings. Numerous recent advances in the application of the physiologic principles to clinical medicine, many of which have been occasioned by the exigencies of war, have necessitated many additions throughout the book. As formerly, the volume is primarily a work on applied physiology, useful not only as a text for medical students but as a reference volume for clinicians.

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*Chemotherapy of Gonococcic Infections. By Russell D. Herrold, B.S., M.D., Associate Professor of Surgery (Urology), College of Medicine, University of Illinois, Chicago, Illinois. Cloth, 137 pages, $3.00. The C. V. Mosby Company, St. Louis, 1943.

†Blood Groups and Transfusion. By Alexander S. Wiener, A.B., M.D., Serologist and Bacteriologist in the Office of the Chief Medical Examiner of New York City; Head of Transfusion Division, The Jewish Hospital of Brooklyn, New York. Cloth, 439 pages, $7.50. Charles C Thomas, Springfield, Ill., Baltimore, Md., 1943.

‡The Physiological Basis of Medical Practice. A University of Toronto Text in Applied Physiology. By Charles Herbert Best, M.A., M.D., D.Sc. (Lond.), F.R.S., F.R.C.P. (Canada); Professor and Head of Department of Physiology; Associate Director of the Connaught Laboratories; Research Associate in the Banting-Best Department of Medical Research, University of Toronto, and Norman Burke Taylor, M.D., F.R.S. (Canada), F.R.C.S. (Edin.), F.R.C.P. (Canada), M.R.C.S. (Eng.), L.R.C.P. (Lond.), Professor of Physiology, University of Toronto. Third Edition. Cloth, 1942 pages, $10.00. A William Wood Book. The Williams & Wilkins Company, Baltimore, 1943.

Allergy, Anaphylaxis and Immunotherapy*

THE contents of Ratner's book, *Allergy, Anaphylaxis and Immunotherapy* is better described in the subtitle, "A Treatise Presenting the Fundamental Principles and Practice Governing the Use of Antisera, Vaccines, Toxoids, Blood Transfusions, Blood Substitutes and Sulfonamides, in the Prevention and Treatment of Infectious Diseases and of the Allergic Phenomena Resulting From Their Use."

It may be described as a book on immunotherapy with homologous and heterologous sera, vaccines, and sulfonamides, with the discussion of allergic and other responses. The analysis of experimental work in the field under discussion, including the author's own contributions, is splendidly arranged and completely up-to-date.

The book will be of great value to allergists, immunologists, serologists, and is especially opportune because of its usefulness to all physicians who have occasion to give whole blood or plasma transfusions.

There is a section dealing with specific immunotherapy in the various infectious diseases.

CORRESPONDENCE

In view of the need for pathologic material in under-graduate and graduate education, the Committee on Pathology of the National Research Council urges that all who have suitable anatomic specimens forward them to the Curator of the Army Medical Museum, Washington, D. C., for correlation and distribution to other central agencies and to teaching institutions. Material from the following is particularly wanted: the malarial diseases, bacillary dysentery, endamebiasis, the schistosomiases, filariasis, the trypanosomiases, the relapsing fevers, the leishmaniases, the rickettsial diseases, yellow fever, cholera, plague, and yaws. On application to the Curator, arrangements for transportation will be made.

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*Allergy, Anaphylaxis and Immunotherapy. Basic Principles and Practice. A Treatise Presenting the Fundamental Principles and Practice Governing the Use of Antisera, Vaccines, Toxoids, Blood Transfusions, Blood Substitutes and Sulfonamides, in the Prevention and Treatment of Infectious Diseases and of the Allergic Phenomena Resulting From Their Use. By Bret Ratner, M.D., Clinical Professor of Pediatrics, New York University College of Medicine; Visiting Pediatrician and Director of Pediatrics, Sea View Hospital; Associate Attending Children's Medical Service, Bellevue Hospital; Consultant Pediatrician, French Hospital. 834 pages, cloth, $8.50. The Williams & Wilkins Company, Baltimore, 1943.